

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.

THIS PAGE BLANK (USPTO)

COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF : Australian Patent
Application 696764 (73941/94). In the name of:
Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by
Ludwig Institute for Cancer Research, under
Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Stuart A. Aaronson of Mount Sinai Medical Center, New York, New York, United States of America, declare as follows:

1. I have been asked by the Patent Attorneys representing Human Genome Sciences ("HGS") to serve as a scientific consultant in connection with the Ludwig Institute for Cancer Research Opposition to the issuance of HGS Australian Patent Application 696764, in the name of HGS, entitled: "Vascular Endothelial Growth Factor-2" ("the HGS patent specification").
2. In acting as a scientific consultant for HGS, I provided a Statutory Declaration executed December 14, 2000 ("Aaronson Declaration I") in connection with the Opposition of the HGS patent specification, in which I provided my comments and opinions on what the HGS patent specification would provide to one skilled in the field of molecular biology of growth factors, *e.g.*, a post doctorate or Ph.D. candidate in a research laboratory, and to provide my comments and opinions on the experimental evidence provided in Dr. Alitalo's Statutory Declaration executed on February 15, 2000 ("Alitalo Declaration I"). I have now been asked by the Patent Attorneys representing HGS to review and provide comments on the Statutory Declaration executed by Dr. Alitalo on September 14, 2001 ("Alitalo Declaration II").

THIS PAGE BLANK (USPTO)

3. The HGS patent specification relates to the identification and characterization of a new member of the PDGF/VEGF family of growth factors, VEGF-2, which is also known as VEGF-C. VEGF-2 and VEGF-C are recognized to be identical proteins by those working in the field, as they have nearly identical sequences as demonstrated by the sequence alignment, appended hereto as Appendix I.
4. In providing my comments and opinions, I have been asked to keep in mind what the HGS patent specification would provide to one familiar with the molecular biology of growth factors as of the earliest filing date of the HGS patent specification, which I have been told is March 1994. For purposes of this analysis, I considered and give an opinion on not only what I knew and appreciated at the relevant time, but also what was expected to be known by one skilled in the field of the molecular biology of growth factors, such as graduate students and postdoctoral fellows who were in my laboratory at the relevant time. The opinions I express are, unless I state to the contrary, opinions based upon these considerations and they would have been applicable as of March 1994, the filing date of the HGS patent specification, as of September 1995, the publication date of the HGS patent specification and are also applicable now. Further, despite the fact that many of my opinions have been presented in the present tense for the ease of expression, I would have held those opinions in March 1994.
5. Dr. Alitalo attempts to provide new experimental data that allegedly addresses the criticisms raised by the HGS scientific consultants of the experimental evidence provided in Alitalo Declaration I. Dr. Alitalo further purports to analyze the expression, proteolytic processing, and secretion profiles of VEGF-2 as taught in the HGS patent specification. However, due to numerous defects in the design, performance, and analysis of these new experiments, the results presented in Alitalo Declaration II fail to provide any

THIS PAGE BLANK (USPTO)

meaningful information regarding the expression, processing and secretion of VEGF-2 as taught in the HGS patent specification. For example, I have identified the following deficiencies in Alitalo Declaration II:

- The failure to follow the teachings of the HGS patent specification as a whole when designing the new experiments;
- The inability to refute that, by following the teachings of the HGS patent specification, the 350 amino acid VEGF-2 protein results in the correct expression, secretion, and processing of the mature form of VEGF-2;
- The failure to design experiments to result in meaningful conclusions with respect to the teachings of the HGS patent specification; and
- The failure to draw credible or consistent conclusions based on the new experiments regarding the expression, processing and secretion of VEGF-2 as taught in the HGS patent specification.

The following paragraphs detail these deficiencies identified in the Alitalo Declaration II.

The Alitalo Declaration II Fails to Follow the Teachings of the HGS Patent Specification as a Whole

6. Dr. Alitalo purports that his new experiments were designed to determine whether utilizing the teachings provided in the HGS patent specification, the 350 amino acid form of VEGF-2 is expressed, processed and secreted. However, the experimental procedures set out in Alitalo Declaration II fail to correct one of the critical flaws of Alitalo Declaration I, *i.e.*, it fails to follow the teachings of the HGS patent specification. In particular, Dr. Alitalo's new experimental design is flawed because he fails to recognize that the HGS patent specification specifically provides that the 350 amino acid sequence may be expressed with a heterologous signal sequence.

THIS PAGE BLANK (USPTO)

7. Likewise, Dr. Alitalo fails to recognize that the experiments described in the Statutory Declaration executed by Susan Powers on December 20, 2000 ("Power Declaration I") demonstrated that VEGF-2 could be expressed, processed and secreted. In particular, Dr. Power's experiments demonstrated that the 350 amino acid form of VEGF-2 can be expressed, processed and secreted from cells when attached to a heterologous signal sequence as specifically taught by the HGS patent specification. (*see* HGS patent specification at page 14, lines 6 to 19).
8. In particular, Dr. Alitalo fails to acknowledge that the HGS patent specification specifically teaches the construction of expression vectors which comprise the 350 amino acid sequence of VEGF-2 fused in frame with a signal sequence. The HGS patent specification provides:

Generally, recombinant expression vectors will include [t]he heterologous structural sequence [is] assembled in appropriate phase with translation initiation and termination sequences, and preferably, *a leader sequence* capable of directing secretion of translated protein into the periplasmic space or extracellular medium. (*see* the HGS patent specification at page 14, lines 6 to 19, emphasis added).

9. Dr. Alitalo has ignored the teaching of the HGS patent specification and has taken the position that no one familiar with the molecular biology of growth factors would utilize a heterologous signal sequence to achieve secretion of a secretory protein which has been identified to have a putative signal sequence. By following the teachings of the HGS patent specification, I believe I or a skilled molecular biologist could utilize a heterologous signal sequence in order to express, process and secrete the 350 amino acid form of VEGF-2.
10. Indeed, contrary to Dr. Alitalo's statements in Alitalo Declaration II, utilizing a heterologous signal sequence is clearly the approach that would be taken by

THIS PAGE BLANK (USPTO)

one familiar with the molecular biology of growth factors. One familiar with the molecular biology of growth factors equipped with the HGS patent specification would recognize that the 350 amino acid polypeptide is a secreted growth factor, and that if the 350 amino acid sequence did indeed contain a signal sequence such a sequence does not have the typical conserved motif of a signal sequence. Thus, if upon expression of the 350 amino acid form of VEGF-2, secretion of the protein did not occur, a skilled molecular biologist would utilize a strong signal sequence to ensure expression and secretion of the protein. Thus, Dr. Alitalo has taken a position which not only is clearly inconsistent with the teachings of the HGS patent specification, but is also contrary to the approach that the skilled molecular biologist would utilize.

11. Dr. Alitalo not only clearly ignores the literal teaching of the HGS patent specification which describes VEGF-2 as a secreted growth factor (*see, e.g.* HGS patent specification at page 5, lines 25 to 33), but incorrectly attributes this recognition to his own research conducted two years subsequent to the filing date of the HGS patent specification (*see* Alitalo Declaration II ¶ 2.2). Contrary to Dr. Alitalo's statements in Alitalo Declaration II, the HGS patent specification filed March 1994 accurately characterizes VEGF-2 as another member of the PDGF/VEGF family of secreted growth factors.
12. In summary, Dr. Alitalo's failure to follow the teachings of the HGS patent specification in designing the experiments provided in Alitalo Declaration II prevents any meaningful conclusions from being drawn about the expression, proteolytic processing and secretion of VEGF-2 as taught in the HGS patent specification.

THIS PAGE BLANK (USPTO)

The Alitalo Declaration II Fails to Refute that by Following the Teachings of the HGS Patent Specification the 350 Amino Acid VEGF-2 Protein Results in the Correct Expression, Secretion, and Processing of the Mature Form of VEGF-2.

13. In my opinion, Dr. Alitalo fails to provide experimental evidence to contradict the fact that the 350 amino acid VEGF-2 fused in frame with a heterologous signal sequence results in the expression, secretion and proteolytic processing of a mature form of VEGF-2.
14. In Alitalo Declaration II, Dr. Alitalo *newly* raises the issue that the HGS patent specification does not provide a description of the molecular weight of the approximately 30 kDa doublet. (see Alitalo Declaration II, ¶5.6).
15. One knowledgeable in the field of molecular biology would not require that the HGS patent specification provide the molecular weight of the secreted form of VEGF-2. Clearly, in following the teachings of the HGS patent specification, as demonstrated by Power Declaration I and Susan Power's Second Declaration ("Power Declaration II"), I or a molecular biologist would recognize that the 350 amino acid sequence of VEGF-2 can be processed to a mature form of VEGF-2 by the cell. Therefore, a molecular biologist provided with the teaching of the HGS patent specification would be able to express VEGF-2 as its naturally processed mature form. Further, a molecular biologist provided with the teaching of the HGS patent specification would recognize that the molecular weight of the resulting processed mature form of VEGF-2 is an intrinsic and natural property of that molecule.
16. Besides molecular weight, the amino acid sequence of a particular protein is also an inherent property of that protein. Statements that Dr Heldin, an apparent colleague of Dr. Alitalo, has made in support of the prosecution of Dr. Alitalo's U.S. Patent No. 6,221,839 are in agreement with this conclusion.

THIS PAGE BLANK (USPTO)

In particular, Dr. Heldin has stated: "It is fundamental biochemistry that polypeptides are organic chemical compounds, albeit sometimes large and complex ones. Like all organic chemical compounds, polypeptides may be characterized by any of *several inherent physical properties, such as molecular formula and molecular weight*. Such physical properties are inherent characteristics of organic molecules in that they are intrinsic properties of the molecules. Because polypeptides are themselves composed of covalently-bonded chains of smaller organic moieties called amino acids (of which there are about 20 naturally occurring), it is conventional to express the molecular formula of polypeptides as an amino acid sequence. *The amino acid sequence of any polypeptide is an inherent property of that polypeptide.*" (see, the Declaration by Dr. Carl-Henrik Heldin, executed June 4, 1997, provided to the U.S. Patent & Trademark Office during prosecution of U.S. Patent No. 6,221,839, the "Heldin Declaration", at page 6, emphasis added).

17. Thus, I or a molecular biologist following the teachings of the HGS patent specification to express the 350 amino acid VEGF-2 polypeptide would recognize that the molecular weight and the amino acid sequence of the naturally processed and secreted form of VEGF-2 is an inherent feature of that polypeptide. Hence, it would be unnecessary for the HGS patent specification to have reported the molecular weight of the 30kDa doublet and 23kDa secreted forms of VEGF-2, because I or a molecular biologist, just like Dr. Heldin, would recognize that the molecular weight and the amino acid sequence is an inherent property of the 350 amino acid form of VEGF-2.
18. Additionally, the Alitalo Declaration II *newly* raises the issue that the processing of the 350 amino acid protein as taught by the HGS patent specification would be incorrect. (see e.g., Alitalo Declaration II ¶ 3.7 or ¶ 5.5).

THIS PAGE BLANK (USPTO)

19. The only information and signals required by a host cell to express and process VEGF-2 to its mature form is contained in the amino acid sequence of VEGF-2. Any given host cell, *i.e.*, a mammalian host cell, will have the proteolytic enzymes and cellular machinery to naturally process VEGF-2 to its mature form. Thus, following the teachings of the HGS specification, the 350 amino acid VEGF-2 polypeptide is naturally and intrinsically processed to its mature form, as demonstrated by the results presented in Power Declaration I and II.
20. I have reviewed and agree with the characterization of the inherent features of the processing of a biologically active mature form of VEGF-2 as provided by Dr. Alitalo himself in portions of the file histories of U.S. Patent Nos. 6,221,839 and 6,245,530.
21. Accordingly, Dr. Alitalo has characterized the processing of VEGF-2 from various cell types, including both mammalian and insect expression systems and has observed that VEGF-2 is intrinsically and naturally processed to its mature form in a wide variety of cell types. (*See*, Declaration by Dr. Kari Alitalo, provided to the US. Patent & Trademark Office on June 10, 2000, in connection with prosecution of U.S. Patent No. 6,245,530, issued June 12, 2001). Evidence of the intrinsic and natural processing of the polypeptide is further confirmed by the observations that the expression of the full length and portions of VEGF-2 in various cell types results in the processing and secretion of a biologically active mature form of VEGF-2. Indeed, Dr. Alitalo has observed that the expression of polypeptides corresponding to residues 104 to 213 and 112 to 419 of the full length VEGF-2 polypeptide are correctly processed to mature forms of VEGF-2 that retain VEGF-2 biological activity. (*See*, Declaration by Dr. Kari Alitalo, provided to the U.S. Patent & Trademark Office on December 1, 1997, in connection with the prosecution of U.S. Patent No. 6,245,530; *see also*, Declaration by Dr. Kari Alitalo, provided to the U.S. Patent & Trademark Office on July 24, 2000, in connection with

THIS PAGE BLANK (USPTO)

the prosecution of U.S. Patent No. 6,221,839; the file histories of both U.S. Patents are annexed hereto as Appendices II and III, respectively).

22. Thus, as Dr. Alitalo has concluded, I or a molecular biologist would also conclude that the full length and portions of the VEGF-2 polypeptide are intrinsically and naturally processed to a biologically active mature form. Likewise, when appropriately expressed as taught by the HGS patent specification, the 350 amino acid form of VEGF-2 contains all of the signals required for the processing of the protein to its biologically active mature form.

The Alitalo Declaration II Fails to Design Experiments that Allows for Meaningful Conclusions with Respect to the Analysis of VEGF-2 as Taught in the HGS Patent Specification.

23. Dr. Alitalo indicates that the experiments reported in Alitalo Declaration II confirm the results of the experiments in Alitalo Declaration I and eliminate any criticisms of the experimental design described in Alitalo Declaration I. However, the criticisms of the experimental design described in Alitalo Declaration II have not been addressed because the experimental design reported in Alitalo Declaration II is also flawed and cannot support any conclusions made with respect to the expression, secretion and processing of VEGF-2 as taught in the HGS patent specification. In particular, the experiments reported in Alitalo Declaration II fail to include experimental controls to address any potential problems with expression vectors, cells, transfection, and conditions and parameters which might affect the comparative analysis of the 350 amino acid VEGF-2 and the 419 amino acid VEGF-2. I discuss these faulty experiments below.
24. Dr. Altilo's conclusions regarding the level of expression of 350 amino acid form of VEGF-2 as compared to the expression of 419 amino acid form of VEGF-2 are meaningless because no experiments were conducted to

THIS PAGE BLANK (USPTO)

determine the transfection efficiency of the plasmids used -- thus preventing the drawing of any valid quantitative comparisons regarding expression efficiencies from the data obtained. Disparate parameters such as cell densities or growth conditions can affect the transfection efficiency of expression constructs into cells. The transfection efficiency will directly correlate with the level of protein expression detected. Clearly if fewer cells contain the construct, fewer cells will express the protein encoded by the construct. Accordingly, any differences in the relative transfection efficiency of the VEGF-2 plasmids utilized in Dr. Alitalo's experiments would affect the comparative detection of levels of VEGF-2 protein. Dr. Alitalo does not provide experiments to demonstrate that following transfection, the same percentage of cells contained each expression construct. In fact, if the transfection efficiency of the 350 amino acid form of VEGF-2 were very low, very few cells would produce protein expressed from the construct and expression and secretion of VEGF-2 protein would be difficult to detect. Without determining transfection efficiency, any conclusion about levels of protein expressed and detected is meaningless.

25. Dr. Alitalo also speculates that the VEGF-2 as taught in the HGS patent specification is not secreted, but rather is rapidly degraded in cells. (see Alitalo Declaration II ¶ 3.9). This speculation has not been affirmatively confirmed by any of the experimental results described in Alitalo Declaration II. Assuming *arguendo* that normally secreted VEGF-2 is rapidly degraded in the cell, then any additional time period that passes before cells are assayed for the presence of VEGF-2 protein would result in the inability to detect the presence of VEGF-2 protein. Furthermore, the experimental protocol described in Alitalo Declaration II does not allow for detection of VEGF-2 protein expression over various time points. Rather, protein levels are assessed fifty hours post-transfection (forty-eight hours and overnight metabolic labeling). Without allowing for the detection of VEGF-2 protein that is purportedly expressed yet degraded with the passage of time over

THIS PAGE BLANK (USPTO)

various periods of time, the conclusions of Dr. Alitalo regarding expression and degradation are merely speculative.

The Alitalo Declaration II Fails to Draw Credible Conclusions with Respect to the Expression, Secretion and Processing of VEGF-2 as Taught in the HGS Patent Specification.

26. In summary, the flaws introduced into the experimental design and protocols of Dr. Alitalo render the experimental results inconclusive. As discussed above, in the absence of appropriate controls, comparative analysis is meaningless because disparate conditions and parameters will affect the expression, secretion and processing profiles of 350 amino acid VEGF-2 and 419 amino acid VEGF-2. In any comparative analysis, results are meaningless without the assurance that unnecessary variables are eliminated. The failure to include basic experimental controls to ascertain that there would be no problems with the expression vectors, cells, transfection efficiency, growth conditions or other parameters which affect any comparative analysis of 350 amino acid VEGF-2 and 419 amino acid VEGF-2 precludes making any meaningful conclusions.

The Alitalo Declaration II Fails to Recognize that the 350 Amino Acid VEGF-2 Is Provided by the HGS Patent Specification

27. Alitalo Declaration II purports to provide a sequence analysis of the VEGF-2 clone deposited with the American Type Culture Collection as ATCC Accession Number 75698. Based on his analysis, Dr. Alitalo alleges that the deposited clone does not have the complete 350 amino acid sequence when compared to the sequence set forth in Figure 1 of the HGS patent specification. However, in my opinion given that Figure 1 of the HGS patent specification does contain the complete 350 amino acid coding sequence, as

THIS PAGE BLANK (USPTO)

even Dr. Alitalo agrees, I fail to see the criticality of Dr. Alitalo's sequence data.

28. Power Declaration II clearly demonstrates that even assuming *arguendo* that the DNA deposited in ATCC Deposit No. 75698 was missing the first 24 amino acids ("the 326 amino acid form of VEGF-2"), both the 350 amino acid form and the 326 amino acid form are processed to the same molecular weight species as compared to the 419 amino acid form of VEGF-2. When expressed as taught by the HGS patent specification, the 350 amino acid form and the 326 amino acid form are both processed to a protein which resolves as a doublet at approximately 30 kDa, as does the 419 amino acid form. (Power Declaration II at ¶ 31). I note that the experimental design of Power Declaration II includes appropriate controls to address transfection efficiencies, sequence confirmation of expression constructs, and controls eliminating any disparities in cell densities or growth conditions, as would be the standard practice of a skilled molecular biologist.
29. Furthermore, the results presented in Power Declaration II clearly evince that even if the DNA deposited in ATCC Deposit No. 75698 did not contain the complete nucleotide sequence provided in Figure 1 of the HGS patent specification, I or a skilled molecular biologist, would have been able to generate the complete 350 amino acid coding sequence based on the nucleotide sequence provided in Figure 1 of the HGS patent specification in combination with standard recombinant techniques known as of March 1994. An example of an approach I could possibly use is the same approach actually used in Power Declaration II, that is, using the sequence provided in Figure 1 of the HGS patent specification, a double stranded oligonucleotide containing the missing sequence is synthesized and is ligated to the DNA obtained from the ATCC, thus recreating the coding sequence of the 350 amino acid form. Alternatively, the template for the PCR reaction could have been obtained by reverse transcription, a technique readily available in March, 1994, using RNA

THIS PAGE BLANK (USPTO)

from sources provided by the HGS patent specification, such as, early stage human embryo osteoclastomas, adult heart or several breast cancer cell lines (see, the HGS patent specification at page 5, lines 19-24, Example 1 and Figure 5). Additionally, I or a skilled molecular biologist, could follow the teachings of the examples provided in the HGS patent specification and obtain the nucleotide sequence encoding either the 419 or the 350 amino acid form of VEGF-2 from a cDNA library derived from early stage human embryo week 9 (see, the HGS patent specification at page 5, lines 19-24).

CONCLUSIONS

30. In sum, the experiments reported in Alitalo Declaration II are not designed to accurately assess the expression of 350 amino acid VEGF-2 as taught in the HGS patent specification. I additionally note that Dr. Alitalo's mischaracterization of the expression profile of VEGF-2 as taught in the application, based on the data generated from experimental design without basis in the teachings of the application is exaggerated by the additional flaws introduced into his experimental protocol. Finally, I note that I am in agreement with Dr. Alitalo's and Dr. Heldin's comments presented in the course of obtaining his own VEGF-2 patents which reflect Dr. Alitalo's recognition that the 350 amino acid VEGF-2 can be naturally processed to its mature form and that the molecular weight and the amino acid sequence of the resulting processed mature form of VEGF-2 are intrinsic and natural properties of that molecule.

AND I declare further that all statements made in this Declaration of my own are true in every particular, and that all statements made on information and belief are believed to be true.

Sworn by the said Dr. Stuart Aaronson, Stuart Aaronson at

New York, New York, on this 22nd day of March 2002;

before me Gean Rotmistrenko

Notary Public

THIS PAGE BLANK (USPTO)

SEQUENCE ANALYSIS DEMONSTRATING VEGF-C AND VEGF-2 ARE THE SAME MOLECULE

VEGF-C	MHLLGFFSVACSLLAALLPGPREAPAAAAAFESGLDLSDAEPDAGEATAYASKDLEEQL	60
VEGF-2	MHSLGFFSVACSLLAALLPGPREAPAAAAAFESGLDLSDAEPDAGEATAYASKDLEEQL	
	*	
VEGF-C	RSVSSVDELMTVLYPEYWKMYKCQLRKGGWQHNREQANLNSRTEETIKFAAAHYNTEILK	120
VEGF-2	RSVSSVDELMTVLYPEYWKMYKCQLRKGGWQHNREQANLNSRTEETIKFAAAHYNTEILK	
VEGF-C	SIDNEWRKTCMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSY	180
VEGF-2	SIDNEWRKTCMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSY	
VEGF-C	LSKTLFEITVPLSQGPKPVTISFANHTSCRCMSKLDVYRQVHSIIRRSIPATLPQCQAAN	240
VEGF-2	LSKTLFEITVPLSQGPKPVTISFANHTSCRCMSKLDVYRQVHSIIRRSIPATLPQCQAAN	
VEGF-C	KTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAGLR	300
VEGF-2	KTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAGLR	
VEGF-C	PASCGPHKELDRNSCQCVCKNKLFP SQGANREFDENTCQCVCKRTCPRNQPLNPGKCAC	360
VEGF-2	PASCGPHKELDRNSCQCVCKNKLFP SQGANREFDENTCQCVCKRTCPRNQPLNPGKCAC	
VEGF-C	ECTESPQKCLLKGGKFHHQTCSCYRRPCTNRQKACEPGFSYSEEVCRCPVSYWKRQMS	419
VEGF-2	ECTESPQKCLLKGGKFHHQTCSCYRRPCTNRQKACEPGFSYSEEVCRCPVSYWQRPMS	

The consensus line:

- * = Indicates substitutions that are neither conserved nor semi-conserved.
- : = indicates conserved substitutions.
- . = indicates semi-conserved substitutions.

AARONSON DECLARATION

APPENDIX I

THIS PAGE BLANK (USPTO)

FILE HISTORY
U.S. PATENT NO. 6,245,530
ISSUED JUNE 12, 2001

1. Application as filed
July 28, 1989
2. Office Action
2/1/90
3. Response to Office Action
5/7/90
4. Information Disclosure Statement
5/7/90
5. Office Action
9/4/90
6. Response to Office Action
2/8/91
7. Office Action
5/3/91
8. Notice of Appeal From the Preliminary Examiner to the Board of Patent Appeals and Interferences
10/31/91
9. Revocation and Appointment of Attorney
11/26/91
10. Power of Attorney to Associate Attorney and Change of Address
4/6/92

THIS PAGE BLANK (USPTO)

14. Information Disclosure Statement
3/21/97
15. Information Disclosure Statement
4/16/97
16. Office Action
5/25/97
17. Amendment and Reply
11/26/97
18. Transmittal of Powers of Attorney/Change of Inventors Address
2/24/98
19. Office Action
3/24/98
20. Amendment and Reply
7/23/98
21. Office Action
10/8/98
22. Supplemental Information Disclosure Statement
7/26/99
23. Supplemental Information Disclosure Statement
10/26/99
24. Office Action
4/4/00
25. Associate Power of Attorney
6/22/00
26. Office Action
6/29/00
27. Amendment and Reply
8/4/00
28. Amendment After Allowance/Request for Approval of Drawing Changes
1/24/01

THIS PAGE BLANK (USPTO)

29. Issue Fee Transmittal
1/24/01

NY2-1223920.1

THIS PAGE BLANK (USPTO)

435	69.4	Class	Subclass	ISSUE CLASSIFICATION		6245530	
UTILITY SERIAL NUMBER		585895		PATENT DATE JUN 12 2007		PATENT NUMBER	
SERIAL NUMBER		08/585,895		FILING DATE		01/12/96	
CLASS		435		SUBCLASS		GROUP ART UNIT	
EXAMINER		52001					

APPLICANT: KARI ALITALO, ESPOO, FINLAND; *Examiner: Wm.*

****CONTINUING DATA*******
 VERIFIED THIS APPLN IS A CIP OF 08/340,011 11/14/94
ExL

****FOREIGN/PCT APPLICATIONS****
 VERIFIED *NONE*
ExL

Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
Verified and Acknowledged		Examiner's Initials						

MARSHALL OTTOLE GERSTEIN MURPHY
 6300 SEARS TOWER
 233 SOUTH WACKER DRIVE
 CHICAGO IL 60606-3402

RECEPTOR LIRAND

U.S. DEPT. OF COMM./PAT. & TM—PTO-436L (Rev.12-94)

04/05/01 Formal Drawings (30 sheets) set L 01/24/01	
PARTS OF APPLICATION FILED SEPARATELY	<i>A. J. Hummel</i> Applications Examiner
NOTICE OF ALLOWANCE MAILED	CLAIMS ALLOWED
18-24-00 12-24-00	Total Claims: 35 Print Claim: 1
ISSUE FEE (W) Amount Due: 1240 Date Paid: 1-20-91	DRAWING Sheets Drwg.: 2430 Figs. Drwg.: 2631 Print Fig.: None
Label Area	ISSUE BATCH NUMBER: 418 PREPARED FOR ISSUE
WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 187 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.	

Form PTO-436A (Rev. 8/92)

Partial Drawings (30 sheets) set

THIS PAGE BLANK (USPTO)

		Date received (Incl. C. of M.) or Date Mailed	Date received (Incl. C. of M.) or Date Mailed
1. Application	papers.		
2.			
3.	Plt of line W. Ontario	Aug. 10, 2000	
34.	Declaration	Aug. 10, 2000	
35.	Amend E	Aug. 10, 2000	
36.	Declaration	8/14/00	
37.	Notice of Motion	10-24-00 1927	
38.	Amend G	01/24/01	
39.	PTOL 271	02/24/01	
40.	Drawings	1/24/01	
41.			
42.			
43.			
44.			
45.			
46.			
47.			
48.			
49.			
50.			
51.			
52.			
53.			
54.			
55.			
56.			
57.			
58.			
59.			
60.			
61.			
62.			
63.			
64.			
65.			
66.			
67.			
68.			
69.			
70.			
71.			
72.			
73.			
74.			
75.			
76.			
77.			
78.			
79.			
80.			
81.			
82.			

(LEFT OUTSIDE)

THIS PAGE BLANK (USPTO)

SEARCHED

Class	Sub	Date	Exmr.
536	23.51	5/25/97	BML
535	23.5		
	25.0.11		
	25.0.12		
	979		
Upd	69.4	3/15/98	ED
Upd	69.4	3/28/00	CL
435	69.4	10/20/00	CL
	70.1		
	325		
	320.1		
536	23.51		
530	399		
985	13		

SEARCH NOTES

	Date	Exmr.
SEE ID NOS. 32 and 33, excerpts attached.	11/15/96	BML
USPAT, MEDLINE, WPIOS searched - see attached.	5/25/97	BML
Update - see search notes in file	3/19/98	KP
seq. search	2/18/00	CL
SEQ ID NO: 32-33	3/28/00	CL
update	10/20/00	CL
update		

INTERFERENCE SEARCHED

Class	Sub	Date	Exmr.
535	69.4	10/20/00	CL
	376.5		
	325		
	320.1		
536	23.51		
530	399		
985	13		

THIS PAGE BLANK (USPTO)

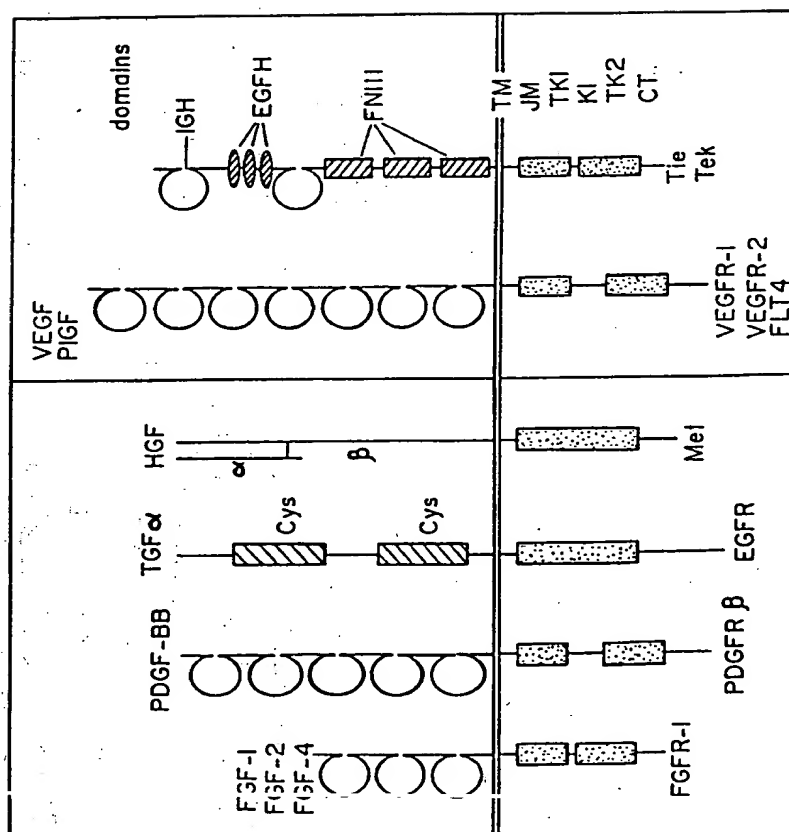


FIGURE 1

THIS PAGE BLANK (USPTO)

FILE HISTORY
U.S. PATENT NO. 6,245,530
ISSUED JUNE 12, 2001

1. Application as filed
July 28, 1989
2. Office Action
2/1/90
3. Response to Office Action
5/7/90
4. Information Disclosure Statement
5/7/90
5. Office Action
9/4/90
6. Response to Office Action
2/8/91
7. Office Action
5/3/91
8. Notice of Appeal From the Preliminary Examiner to the Board of Patent Appeals and Interferences
10/31/91
9. Revocation and Appointment of Attorney
11/26/91
10. Power of Attorney to Associate Attorney and Change of Address
4/6/92

THIS PAGE BLANK (USPTO)

14. Information Disclosure Statement
3/21/97
15. Information Disclosure Statement
4/16/97
16. Office Action
5/25/97
17. Amendment and Reply
11/26/97
18. Transmittal of Powers of Attorney/Change of Inventors Address
2/24/98
19. Office Action
3/24/98
20. Amendment and Reply
7/23/98
21. Office Action
10/8/98
22. Supplemental Information Disclosure Statement
7/26/99
23. Supplemental Information Disclosure Statement
10/26/99
24. Office Action
4/4/00
25. Associate Power of Attorney
6/22/00
26. Office Action
6/29/00
27. Amendment and Reply
8/4/00
28. Amendment After Allowance/Request for Approval of Drawing Changes
1/24/01

THIS PAGE BLANK (USPTO)

29. Issue Fee Transmittal
1/24/01

THIS PAGE BLANK (USPTO)

435 69.4		Class	Subclass	ISSUE CLASSIFICATION	6245530	
UTILITY SERIAL NUMBER	585895	PATENT DATE JUN 12 2001		PATENT NUMBER 6245530		
SERIAL NUMBER	08/585,895	FILING DATE	01/12/96	CLASS	435	GROUP ART UNIT
				SUBCLASS		EXAMINER

APPLICANT: KARI ALITALO, ESPOO, FINLAND; Attorney, USA

CONTINUING DATA***
 VERIFIED THIS APPLN IS A CIP OF 02/340,011 11/14/94
BRL AND

FOREIGN/PCT APPLICATION INFORMATION
 VERIFIED NONE
BRL

Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
Verified and Acknowledged	Examiner's Initials							

MAILED: MARSHALL D'ONOFIO GERSTEIN MURPHY & ASSOCIATES
 6300 SEARS TOWER
 233 SOUTH WACKER DRIVE
 CHICAGO IL 60606-6402

RECEPTOR LOGAND

U.S. DEPT. OF COMM./PAT. & TM—PTO-436L (Rev.12-94)

04/05/01		Formal Drawings (30 shts) set L		01/24/01	
PARTS OF APPLICATION FILED SEPARATELY				Applications Examiner	
NOTICE OF ALLOWANCE MAILED		Assistant Examiner		CLAIMS ALLOWED	
18-24-00				Total Claims	Print Claim
12-24-00				35	1
ISSUE FEE (W)		CHRISTINE GAOUO PATENT EXAMINER		DRAWING	
Amount Due	Date Paid			Sheets Drwg.	Figs. Drwg.
12-40	1-20-01			2430	2631
Label Area		Christine Gaouo Primary Examiner		Print Fig.	None
		PREPARED FOR ISSUE		ISSUE BATCH NUMBER	U18
WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 187 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.					

085895

Model 1800

PATENT APPLICATION



08585895

Date Entered or Counted

CONTENTS *Hq*

APPROVED FOR LICENSE

INITIALS

Date Received or Mailed

1. Application *24 Sheets papers.*

2. *Raw Sequence Listing (OK)*

3. *Lab. Declaration / Fee*

4. *Petition (S3)*

5. *Petition granted (S3)*

6. *Best. 30 days*

7. *Pre Amt / A*

8. *I.O.S. w/ Attch*

9. *I.D.S.*

10. *PETITION TO EXPEDITE (PETITION)*

11. *I.D.S.*

12. *I.D.S.*

13. *I.D.S.*

14. *Lab. Declaration / Fee*

15. *Declaration / Amendment / Fee*

16. *INT. DISC. AMT*

17. *REI 3 mos*

18. *Ext. of Time (3 months)*

19. *Amendment / Declaration / Fee / Disk*

20. *Power of Attorney*

21. *Raw Sequence Listing*

22. *Lab. 3 mos*

23. *Declaration*

24. *Ext. of Time 1 month*

25. *Lab. 3 mos*

26. *Amendment - D*

27. *Letter of Suspension*

28. *S.I.D.*

29. *S.I.D.S.*

30. *Req. 3 months*

31. *Power of Attorney*

32. *Interview Summary*

Amend. Inside (FRONT)

8/16/96
APR 14 1996

5/24/96

7/22/96

11/25/96

8-14-96

11-1-96

12-23-96

06-25-96

02-11-97

03-24-97

1/23/97

7-12/1997

1/27/97

4/21/97

5-28-97

12/01/97

12/01/97

02/26/98

01/22/98

13-24-98

7/23/98

7-27-98

7-27-98

7-27-98

10/8/98

7-28-99

Oct. 28, 1999

4/10/2000

10-22-00

6-29-00

		Date received (Incl. C. of M.) or Date Mailed	Date received (Incl. C. of M.) or Date Mailed
1. Application	papers.		
2.			
3.			
33.	Photo of Jane W. Wronski	Aug. 10, 2000	
34.	Declaration	Aug. 10, 2000	
35.	Amclt E	Aug. 10, 2000	
36.	Declaration	8/14/00	
37.	Amclt of Monroe	10-24-00 1927	
38.	Amclt G	01/24/01	
39.	PTOL 271	02/24/01	
40.	Drawings	1/24/01	
41.			
42.			
43.			
44.			
45.			
46.			
47.			
48.			
49.			
50.			
51.			
52.			
53.			
54.			
55.			
56.			
57.			
58.			
59.			
60.			
61.			
62.			
63.			
64.			
65.			
66.			
67.			
68.			
69.			
70.			
71.			
72.			
73.			
74.			
75.			
76.			
77.			
78.			
79.			
80.			
81.			
82.			

(LEFT OUTSIDE)

POSITION	ID NO.	DATE
CLASSIFIER		
EXAMINER	4/6	2-22-96
TYPIST		4/15/96
VERIFIER		
CORPS CORR.		
SPEC. HAND	509	7-11-96
FILE MAINT.	323	5-14
DRAFTING		

INDEX OF CLAIMS

Claim	Final	Original	Date
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			
32			
33			
34			
35			
36			
37			
38			
39			
40			
41			
42			
43			
44			
45			
46			
47			
48			
49			
50			

SYMBOLS
✓ Rejected
- Allowed
(Through number) Cancelled
N Restricted
I Non-elected
A Interference
O Appeal
O Objection

Claim	Final	Original	Date
51			
52			
53			
54			
55			
56			
57			
58			
59			
60			
61			
62			
63			
64			
65			
66			
67			
68			
69			
70			
71			
72			
73			
74			
75			
76			
77			
78			
79			
80			
81			
82			
83			
84			
85			
86			
87			
88			
89			
90			
91			
92			
93			
94			
95			
96			
97			
98			
99			
100			

SEARCHED

Class	Sub	Date	Exmr.
536	23.51	5/25/97	BML
535	325		
	325/11		
	325/12		
	325		
433	69.4	3/15/98	CP
	70.1	3/23/00	CL
	325	10/20/00	CL
	320.1		
536	23.51		
530	399		
935	13		

SEARCH NOTES

	Date	Exmr.
SEQ ID NOS 32 and 33, excerpts attached.	11/15/96	BML
USPAT, MEDLINS, WPIOS searched - see attached.	5/25/97	BML
Update - see search notes in file	3/19/98	KP
seq. search	2/10/00	CL
SEQ ID NO: 32-33	3/25/00	CL
update	10/20/00	CL
update		

INTERFERENCE SEARCHED

Class	Sub	Date	Exmr.
35	69.4	10/20/00	CL
	70.1		
	325		
	320.1		
36	23.51		
324	399		
35	13		



US006245530B1

(12) **United States Patent**
Alitalo et al.

(10) Patent No.: **US 6,245,530 B1**
(45) Date of Patent: **Jun. 12, 2001**

(54) **RECEPTOR LIGAND**

(75) Inventors: Kari Alitalo, Espoo (FI); Vladimir Joukov, Boston, MA (US)

(73) Assignees: Ludwig Institute for Cancer Research, New York, NY (US); Helsinki University Licensing, Ltd. OY, Helsinki (FI)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 08/585,895

(22) Filed: Jan. 12, 1996

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/510,133, filed on Aug. 1, 1995.

(51) Int. Cl.⁷ C12N 15/12; C12N 15/63; C12N 5/10; C12N 5/16

(52) U.S. Cl. 435/69.4; 435/70.1; 435/325; 435/320.1; 536/23.51; 530/399; 935/13

(58) Field of Search 536/23.51; 435/252.3; 435/254.11; 320.1; 419; 69.4; 70.1; 325; 530/399; 935/13

(56) **References Cited****U.S. PATENT DOCUMENTS**

5,219,739 6/1993 Tischer et al. 435/69.4
5,332,671 7/1994 Ferrara et al. 435/240.1
5,607,918 3/1997 Eriksson et al. 514/12
5,932,540 8/1999 Jing-Shan Hu et al.
5,935,820 8/1999 Jing-Shan Hu et al.

FOREIGN PATENT DOCUMENTS

0 506 477 A1 3/1992 (EP)
WO 95/24473 A1 9/1995 (WO)
WO 95/33050 12/1995 (WO)
WO 95/33772 12/1995 (WO)
WO 96/11269 A2 4/1996 (WO)
WO 96/30046 10/1996 (WO)
WO 96/39421 12/1996 (WO)
WO 96/39515 12/1996 (WO)
97/05250 2/1997 (WO)
97/09427 3/1997 (WO)
97/17442 5/1997 (WO)

OTHER PUBLICATIONS

Reeck et al. Cell 50, 667, 1987.*
Hillier et al. y185b08.21 *Homo sapiens* cDNA clone 44993 5' EST-STS Accession No. H05177, Jun. 21, 1995.*
Hillier et al. y186g06.r1 *Homo sapiens* cDNA clone 45138 5' EST-STS Accession No. H07991, Jun. 23, 1995.*
Hillier et al. yd29f07.r1 *Homo sapiens* cDNA clone 109669 5' similar to SP:BAR3_CHITE Q03376 Balbiani Ring Protein 3, EST-STS Accession No. T81690, Mar. 15, 1995.*
Auffray et al. *H. sapiens* partial cDNA sequence: clone c-1wf11, EST-STS Accession No. Z44272, Nov. 6, 1994.*

Pajusola. "Cloning and Characterization of a New Endothelial Receptor Tyrosine Kinase Flt4 and Two Novel VEGF-Like Growth Factors VEGF-B and VEGF-C." Academic Dissertation, Molecular/Cancer Biology Laboratory and Department of Pathology, Haartman Institute and Department of Biosciences, Division of Genetics, University of Helsinki, (Jan. 26, 1996).

Hillier et al., "The WashU-Merck EST Project." EMBL Database entry HS991157, accession No. H07991, Jul. 2, 1995.

Alitalo et al., "Vascular Endothelial Growth Factors and Receptors Involved in Angiogenesis," *The 9th International Conference of the International Society of Differentiation (ISD), Development, Cell Differentiation and Cancer*, Pisa (Italy), Sep. 28-Oct. 2, 1996, p. 66 (Abstract S22).

Alitalo et al., "Vascular Endothelial Growth Factors B and C and Receptors Involved in Angiogenesis," *German-American Academic Council Foundation (GAACF) Stiftung Deutsch-Amerikanisches Akademisches Konzil (DAAK), 2nd Symposium on Current Problems in Molecular Medicine: The Role of Cytokines in Human Disease*, Nov. 17-20, 1996, Ringberg Castle, Germany, p. 1 (Abstract).

Kukk et al., "VEGF-C Receptor Binding and Pattern of Expression with VEGFR-3 Suggests a Role in Lymphatic Vascular Development," *Development*, 122:3829-3837 (1996).

Paavonen et al., "Chromosomal Localization and Regulation of Human Vascular Endothelial Growth Factors B and C (VEGF-B and VEGF-C)," *IX International Vascular Biology Meeting*, Seattle, Washington, Sep. 4-8, 1996, p. 76 (Abstract 299).

U.S. application No. 08/207,550, Jing-Shan Huand Liang Cao, Mar. 8, 1994.

U.S. application No. 08/465,968, Crain Rosen, Jing-Shan Hu and Liang Cao, Jun. 6, 1995.

Provisional application No. 60/003,491, James Lee and William Wood, Sep. 8, 1995.

U.S. application No. 08/554,374, Lyman, S., Nov. 8, 1995.

Achen, M.G. et al., "Vascular Endothelial Growth Factor D (VEGF-D) is a Ligand for the Tyrosine Kinases VEGF Receptor 2 (Flk1) and VEGF Receptor 3 (Flt4)," *Proceedings of the National Academy of Science, USA*, 95:548-553 (Jan., 1998).

Adams, M.D. et al., "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence," *Nature*, 377(6547 Supplement):3-174 (Sep., 1995).

(List continued on next page.)

Primary Examiner—Christine Saoud
(74) Attorney, Agent, or Firm—Marshall, O'Toole, Gerstein, Murray & Borun

(57) **ABSTRACT**

Provided are ligands for the receptor tyrosine kinase, Flt4. Also provided are cDNAs and vectors encoding the ligands, pharmaceutical compositions and diagnostic reagents.

35 Claims, 30 Drawing Sheets

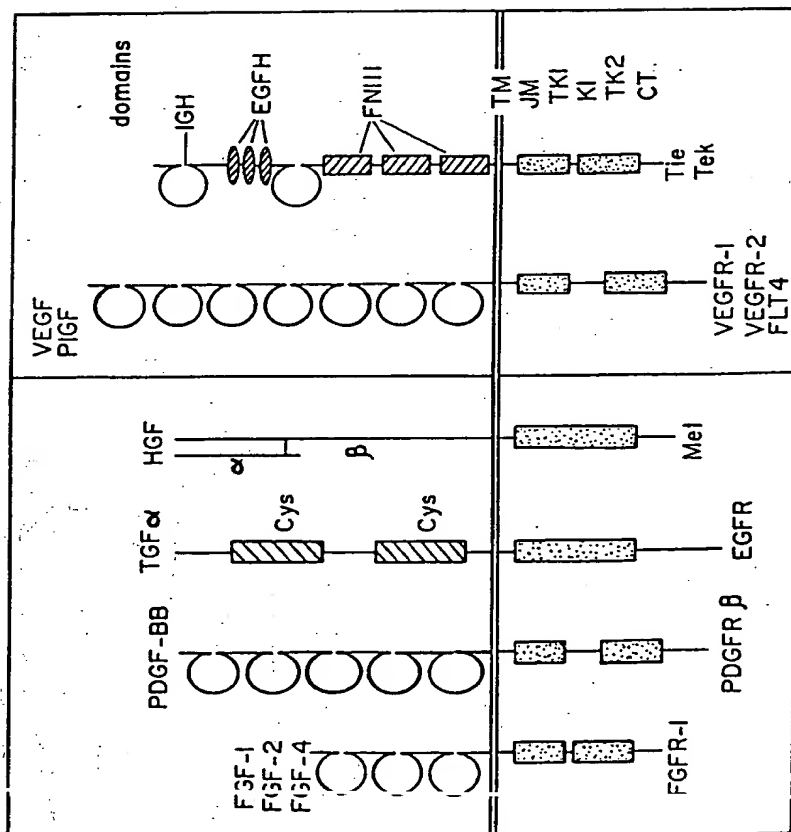
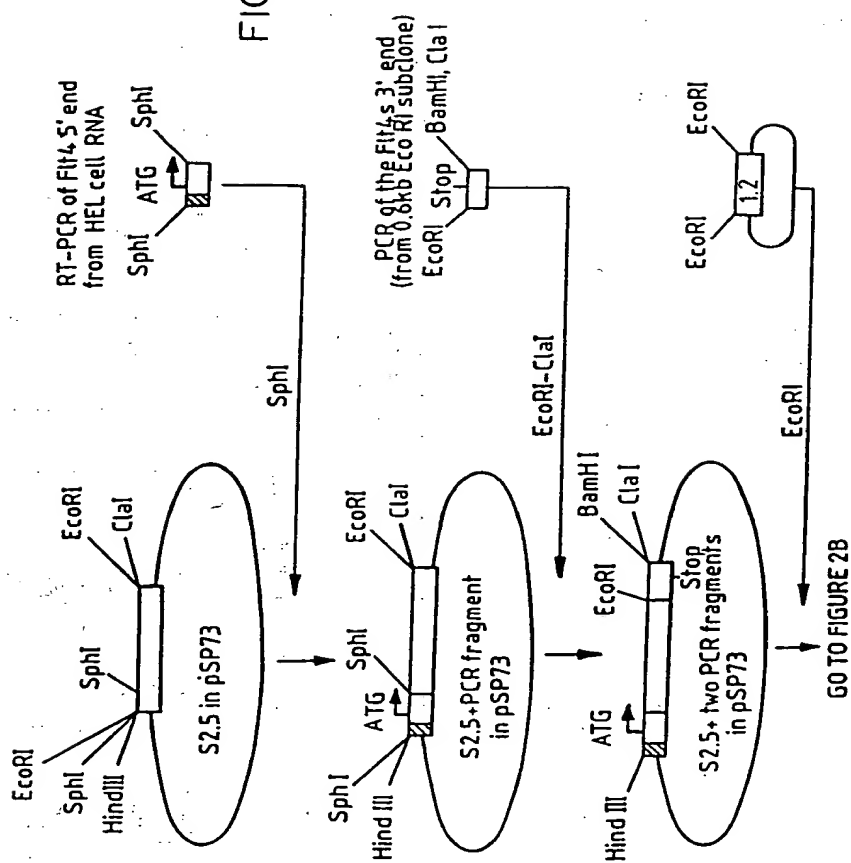
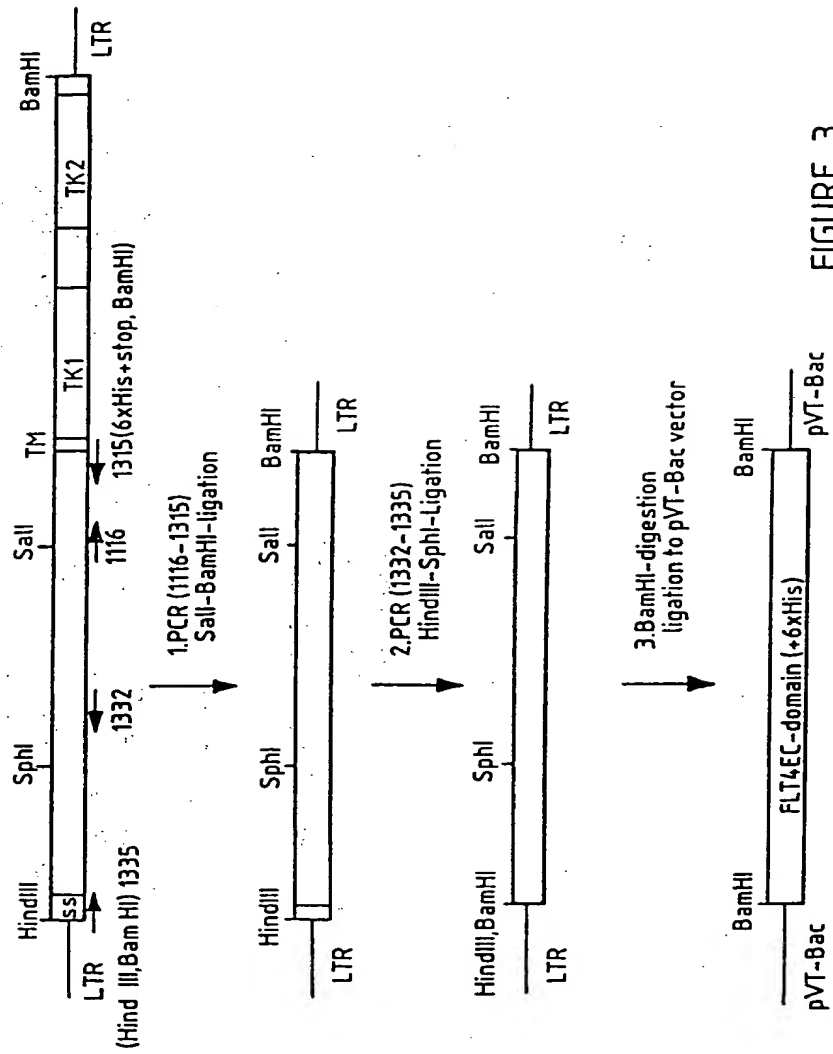


FIGURE 1

FIGURE 2A



[illegible]



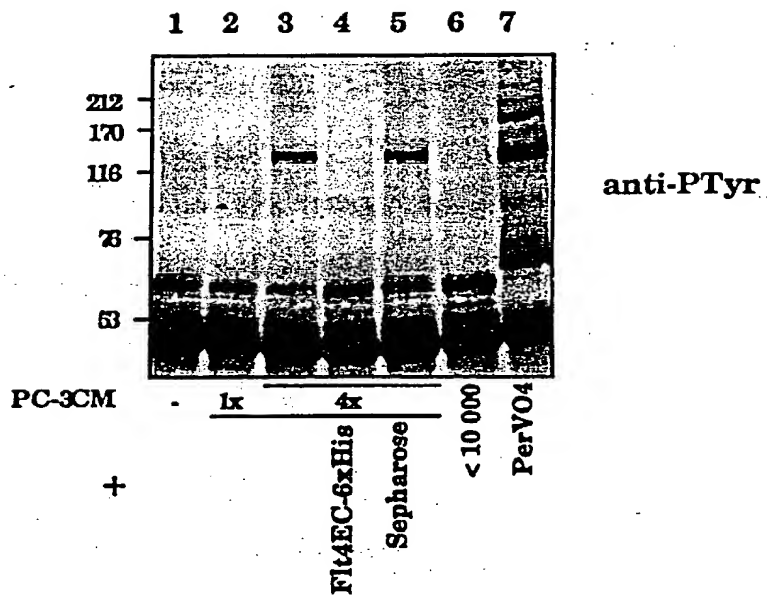
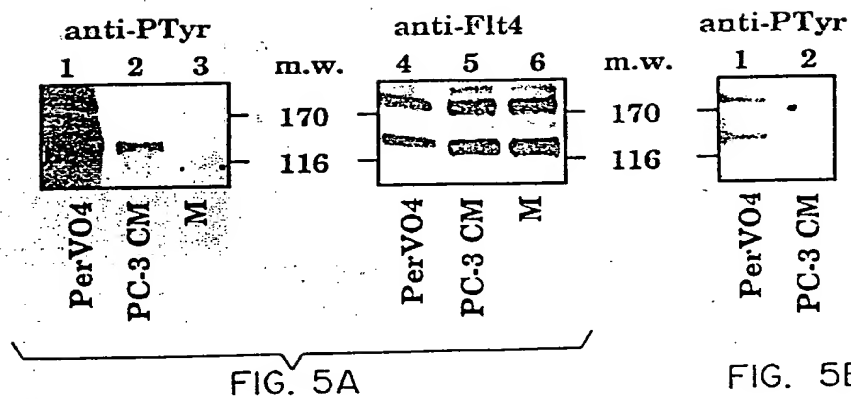


FIG. 4



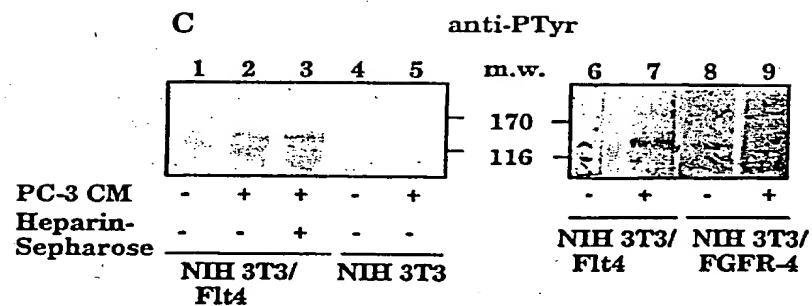


FIG. 6

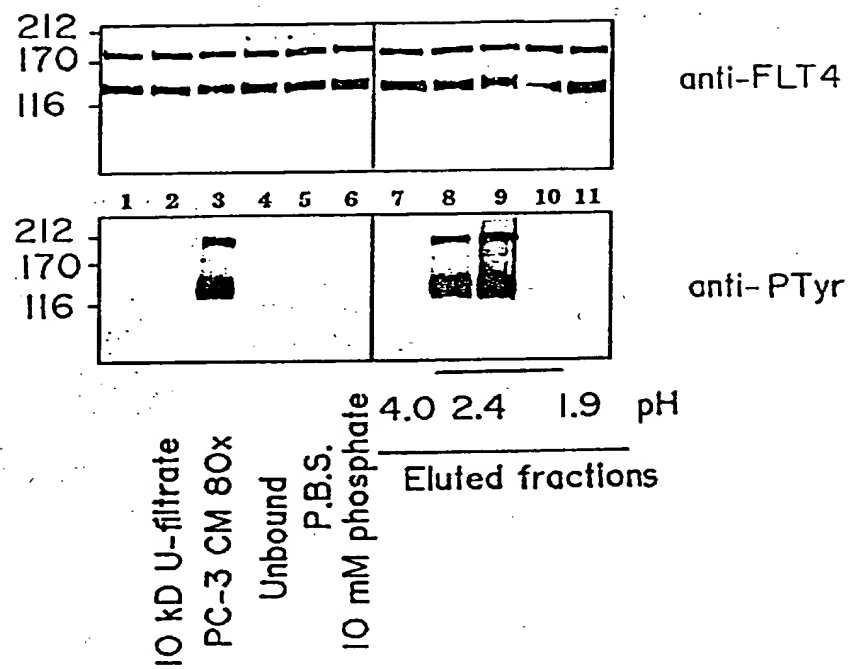
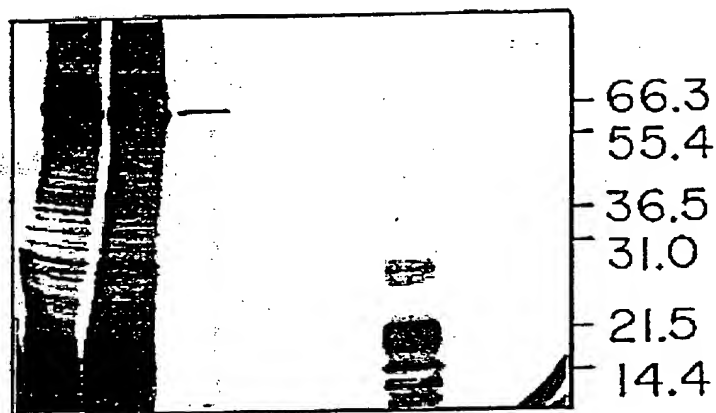


FIG. 7



PC-3 CM 80x

Unbound

P.B.S.

10 mM phosphate

4.0 2.4 1.9

Eluted
fractions

M.w. standards

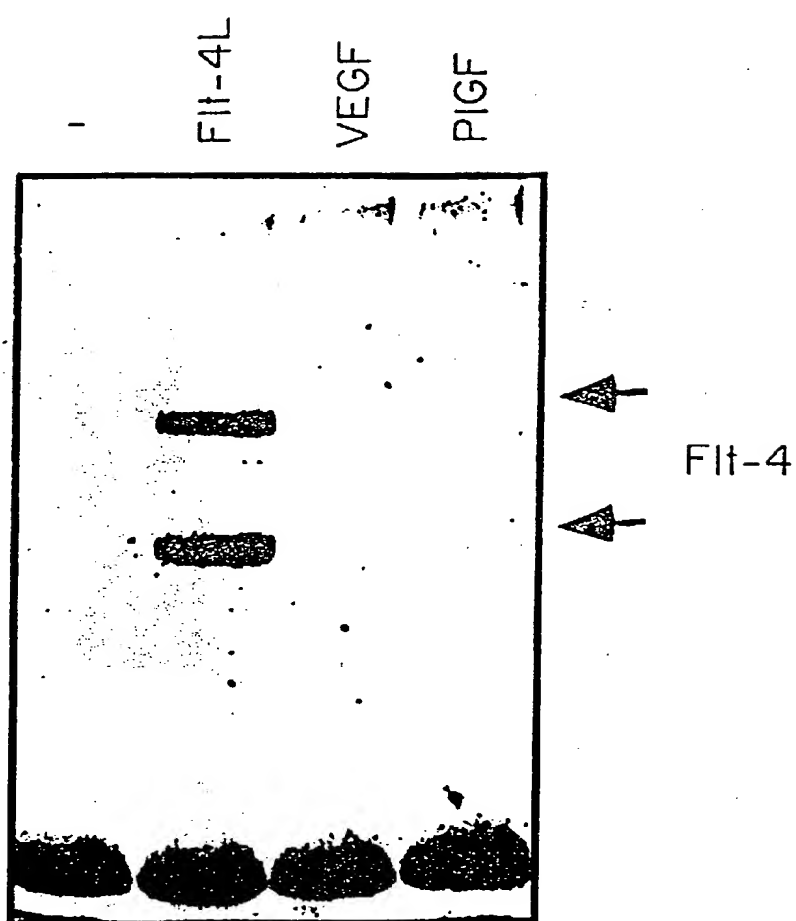


FIG. 8

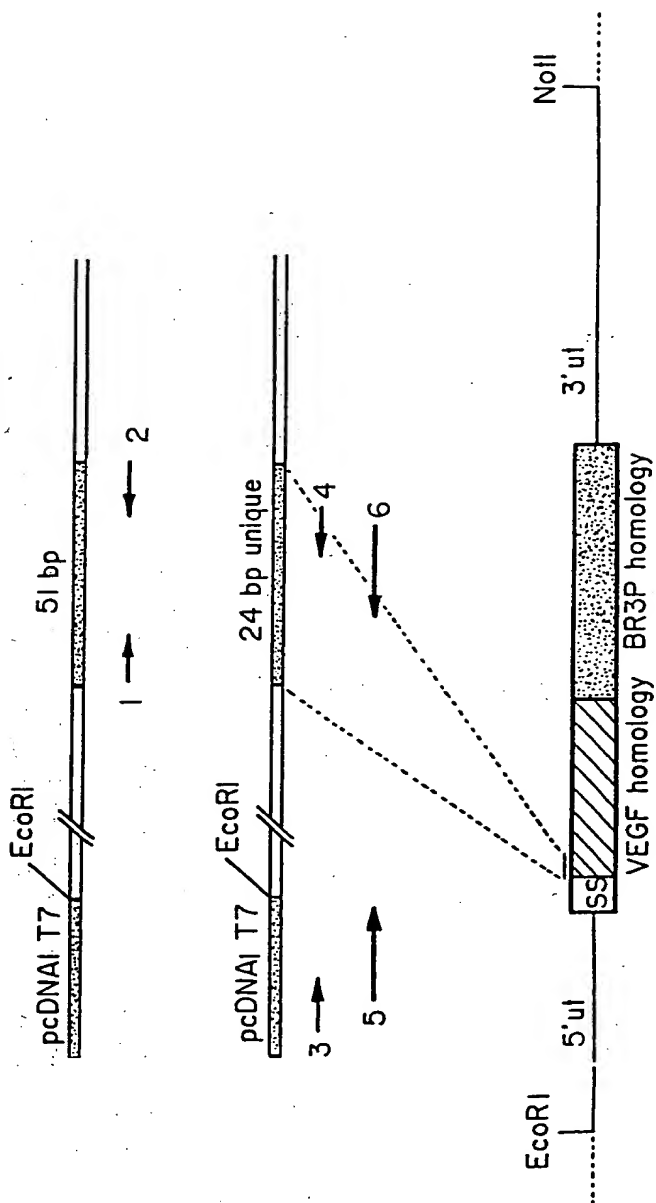


FIG. 9A

MetThrValLeuTyrProGluTyr
GAGCAGTTACGGTCTGTGTCAGTGTAGTGAACCTCATGCTACTCTACCCAGAATAT 50
10 30

TrpLysMetTyrLysCysGlnLeuArgLysGlyGlyTrpGlnHisAsnArgGluGlnAla
TGGAAAATGTACAAGTGTCAAGTGTCAAGTAAGGAAAGGAGGCTGGCAACATAACAGAGAACAGGCC 110
70 90

AsnLeuAsnSerArgThrGluGluThrIleLysPheAlaAlaAlaHisTyrAsnThrGlu
AACCTCAACTCAAGGACAGAGAGACTATAAAATTTGCTGCAGCACATTATATAATACAGAG 170
130 150

IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys
ATCTTGAAAAGTATTGATAATGAGTGGAGAAAGACTCAATGCATGCCACGGGAGGTGTGT 230
190 210

IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProProCysVal
ATAGATGTGGGAAGGAGTTTGGAGTCGCGACAAACACCTTCTTTAAACCTCCATGTGTG 290
250 270

SerValTyrArgCysGlyCysCysAsnSerGluGlyLeuGlnCysMetAsnThrSer
TCCGCTCTACAGATGTGGGGTGTGCTGCAATAGTGGGGGCTGCAGTGCATGAACACCAGC 350
310 330

FIG. 9B

ThrSerTyrLeuSerLysThrLeuPheGluIleThrValProLeuSerSerGlnGlyProLys
ACGAGCTACCTCAGCAAGACGTTATTGAAATTACAGTGCCTCTCTCTCAAGGCCCCCAA
370 390 410

ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal
CCAGTAACAATCAGTTTGGCCAATCACACTTCCTGCCGATGCATGTCTAAACTGGATGTT
430 450 470

TyrArgGlnValHisSerIleIleArgArgSerLeuProAlaThrLeuProGlnCysGln
TACAACAAGTTCAATCCATTATTAGACGTTCCCTGCCAGCAACACTACCACAGTGTCTAG
490 510 530

AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu
GCAGTGAACAAGACCTGCCCCCACCATTACATGTGGAAATATCACATCTGCAGATGCCTG
550 570 590

AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspAspSerThrAspGlyPheHis
GCTCAGGAAGATTTTATGTTTTCTCGGATGCTGGAGATGACTCAACAGATGGATTCCAT
610 630 650

AspIleCysGlyProAsnLysGluLeuAspGluThrCysGlnCysValCysArgAla
GACAICTGTGGACCAACAAGGAGCTGGATGAAGACCTGTCTAGTGTGTCTGCAGAGCG
670 690 710

GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys
GGGCTTCGGCCTGCCAGCTGTGGACCCCAAGAACTAGACAGAACTCATGCCAGTGT
730 750 770

FIG. 9C

ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAsn
GTCTGTAAACAAACTCTTCCCCAGCCAATGTGGGCCCAACCGAGAATTGTATGAAAC
790 810 830

ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys
ACATGCCAGTGTGTATGTAAAGAACCTGCCCCAGAAATCAACCCCTAAATCCTGGAAAA
850 870 890

CysAlaCysGluCysThrGluSerProGlnLysCysLeuLysGlyLysLysPheHis
TGTGCTGTGAATGTACAGAAAGTCCACAGAAATGCTTGTAAAGGAAAGAAGTTCAC
910 930 950

HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro
CACCAACATGCAGCTGTTACAGACGGCCATGTACGAACCCGCCAGAGGCTTGTGAGCCA
970 990 1010

GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln
GGATTTTCATATAGTGAAGAAGTGTGCTGTGTCCCTTCATATTTGGAAAAGACCACAA
1030 1050 1070

MetSerEnd
ATGAGCTAAGATTGTACTGTTTTCCAGTTCATCGATTTTCTATTATGGAAAACTGTGTTG
1090 1110 1130

FIG. 9D

1	PDGF-A	.MRTWACLLL	LCCGYLAHAL	AEAEIPREL	IERLARSQIH	SIRDLQRILLE	50
	PDGF-B	MNRCWA.LFL	SLCCYLRVLS	AEGDPIPEEL	YEMLSHSIR	SFDDLQRLLH	
	PlGF-1	MPVM	RLFPC..	FLQ LLAGLAL...	
	PlGF-2	MPVM	RLFPC..	FLQ LLAGLAL...	
	VEGF121	M NFLLS..	WVH WSLALLLYLH	
	VEGF165	M NFLLS..	WVH WSLALLLYLH	
	VEGF189	M NFLLS..	WVH WSLALLLYLH	
	VEGF206	M NFLLS..	WVH WSLALLLYLH	
	FLT4-L	M TVLYPEYWK	M YKQRLRKGGW	
51	PDGF-A	IDSVGAEDAL	ETSLRAHGSH	AINHVPEKRP	VPIRRKRSI.EEAIP	100
	PDGF-B	GDP.GEEDGA	ELDLNMTRSH	SGGELES...	.LARGRRSLG	SLTIAEPAMI	
	PlGF-1	PAVPPQQW..ALSAG	NGSSEVEVVP	FQE.VWGR..	
	PlGF-2	PAVPPQQW..ALSAG	NGSSEVEVVP	FQE.VWGR..	
	VEGF121	HAKWSQAA..PMAEG	GGQNHHEVVK	FMD.VYQR..	
	VEGF165	HAKWSQAA..PMAEG	GGQNHHEVVK	FMD.VYQR..	
	VEGF189	HAKWSQAA..PMAEG	GGQNHHEVVK	FMD.VYQR..	
	VEGF206	HAKWSQAA..PMAEG	GGQNHHEVVK	FMD.VYQR..	
	FLT4-L	QHNREQANLN	SRTEETIKFA	AAHYNTEILK	SIDNEWK...	

FIG. 10A

101	150
PDGF-A	AVCKTRTVIY EIPRSQVDPT SANFLIWPPC VEVKRCTGCC NTSSVKCQPS
PDGF-B	AECKTRTEVF EISRRLLDRT NANFLVWPPC VEVQRCGSCC NNRNVQCRPT
P1GF-1	SYCRALERLV DWVSEYPS.. EVEHMFSPSC VSLLRCTGCC GDENLHCVPV
P1GF-2	SYCRALERLV DWVSEYPS.. EVEHMFSPSC VSLLRCTGCC GDENLHCVPV
VEGF121	SYCHPIETLV DIFQEYPD.. EIEYIFKPPC VPLMRGCGCC NDEGLECVPT
VEGF165	SYCHPIETLV DIFQEYPD.. EIEYIFKPPC VPLMRGCGCC NDEGLECVPT
VEGF189	SYCHPIETLV DIFQEYPD.. EIEYIFKPPC VPLMRGCGCC NDEGLECVPT
VEGF206	SYCHPIETLV DIFQEYPD.. EIEYIFKPPC VPLMRGCGCC NDEGLECVPT
FLT4-L	TQCMPEVCI DVGKEFGV.. ATNTFFKPPC VSVYRCGGCC NSEGLQCMNT
200	
PDGF-A	RVHHRSVKVA KVEYVRKKPK LKEVQVRLEE HLEACAT..
PDGF-B	QVQLRPVQVR KIEIVRKKPI FKKATVTLED HLACKCETVA AARPVTRSPG
P1GF-1	ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRCECRPLR EKMKPER...
P1GF-2	ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRCECRPLR EKMKPERRR.
VEGF121	EESNITMQIM RIKPH..QGQ .HIGEMSFLQ HNKECPRPK DRARQEKCD.
VEGF165	EESNITMQIM RIKPH..QGQ .HIGEMSFLQ HNKECPRPK DRARQEN...
VEGF189	EESNITMQIM RIKPH..QGQ .HIGEMSFLQ HNKECPRPK DRARQEKKS.
VEGF206	EESNITMQIM RIKPH..QGQ .HIGEMSFLQ HNKECPRPK DRARQEKKS.
FLT4-L	STSYLSKTLF EITVPLSQGP .KPVTIISFAN HTSCRCMSKL DVYRQVHSII

FIG. 10B

FIG. 10C

301	PDGF-A	350
	PDGF-B	
	PlGF-1	
	PlGF-2	
	VEGF121	
	VEGF165	
	VEGF189	
	VEGF206	
	FLT4-L	SQCGANREFD	ENTCQCVCCKR TCPRNQPLNP GKCACTES PQKCLLKGGK	
351	PDGF-A	395
	PDGF-B	
	PlGF-1	
	PlGF-2	
	VEGF121	
	VEGF165	
	VEGF189	
	VEGF206	
	FLT4-L	FHHQTCSCYR	RPCTNRQKAC EPGFSYSEEV CRCVPSYWKR PQMS	

FIG. 10D

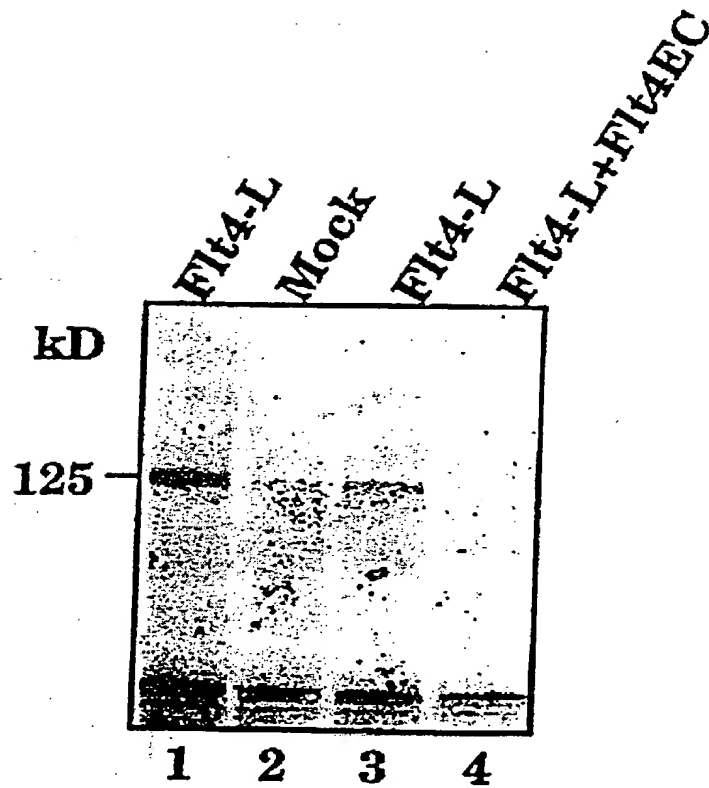


FIG. II

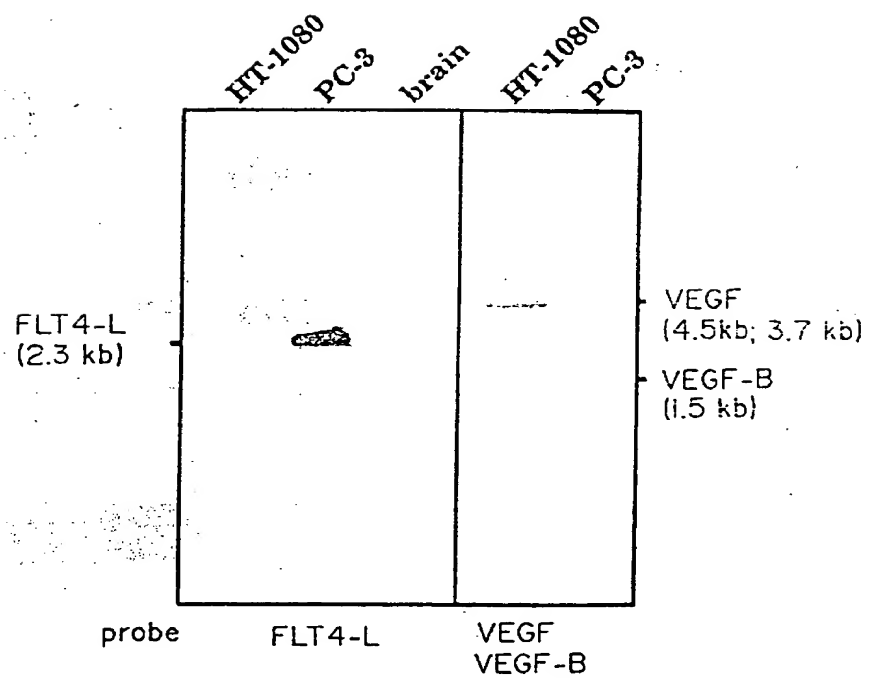


FIG. 12

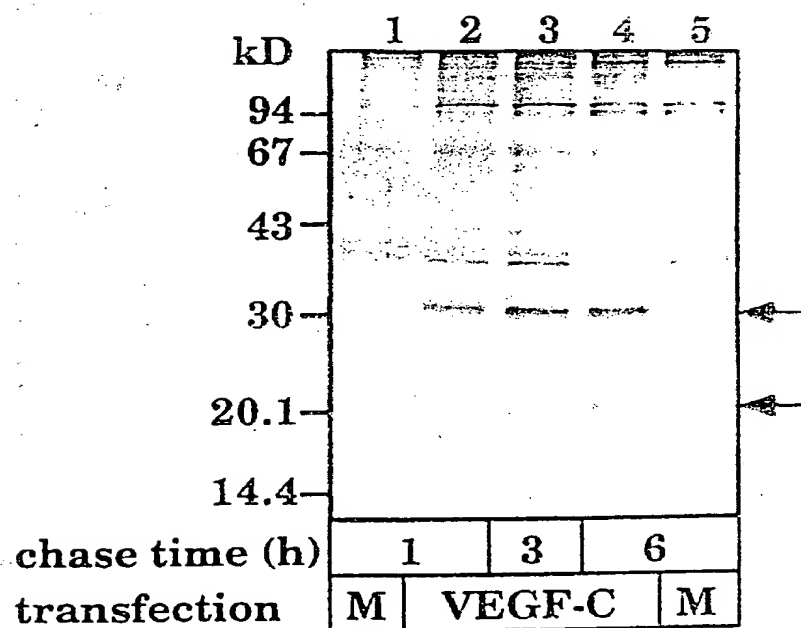


FIG. 13A

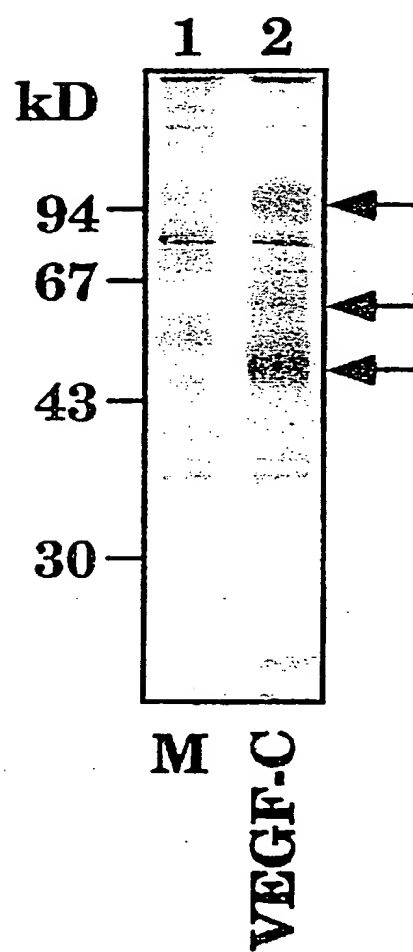


FIG. 13B

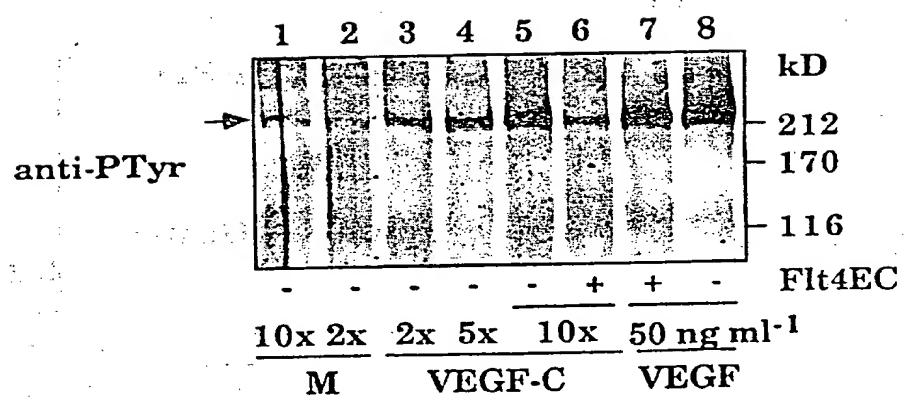


FIG. 14A

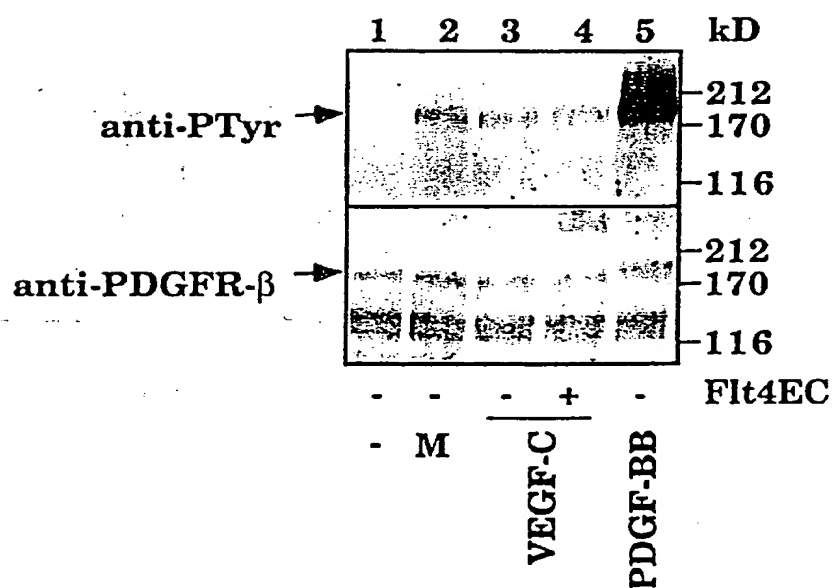
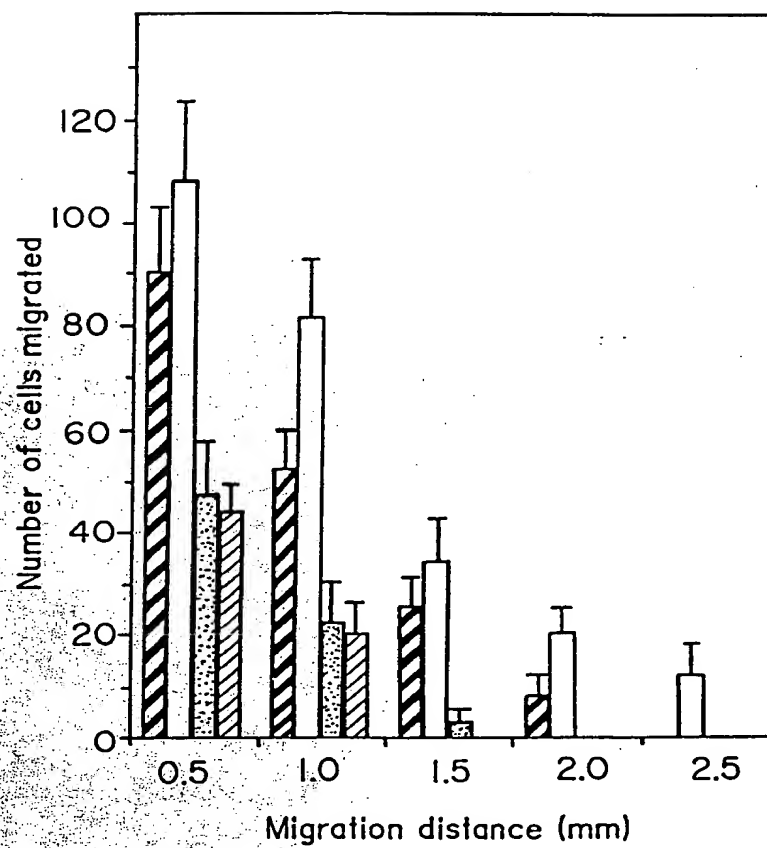


FIG. 14B



▨ VEGF -C
□ VEGF
▤ MOCK
▧ CONTROL

FIG. 15A

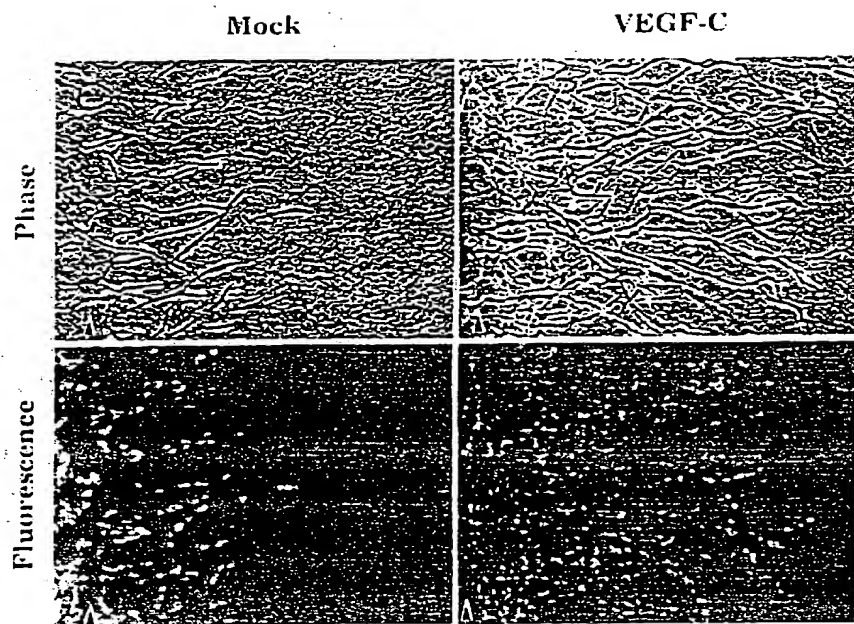


FIG. 15B

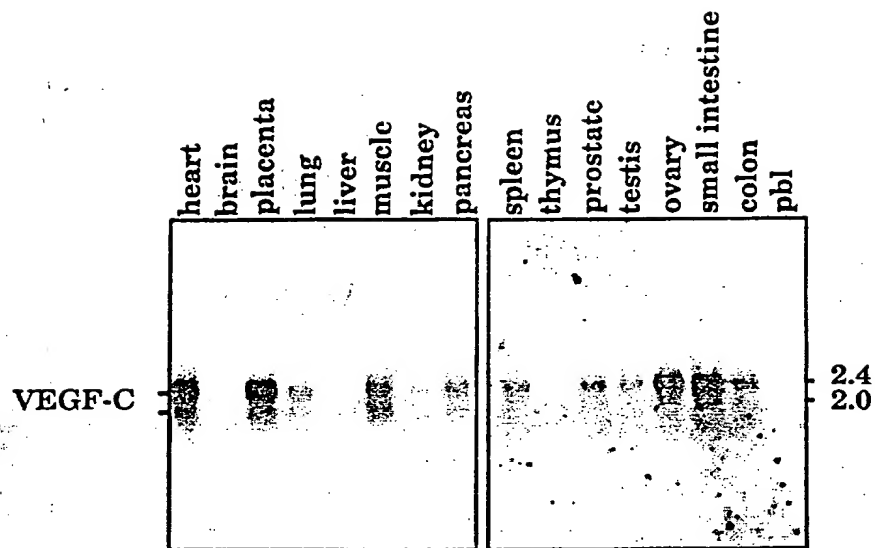


FIG. 16A

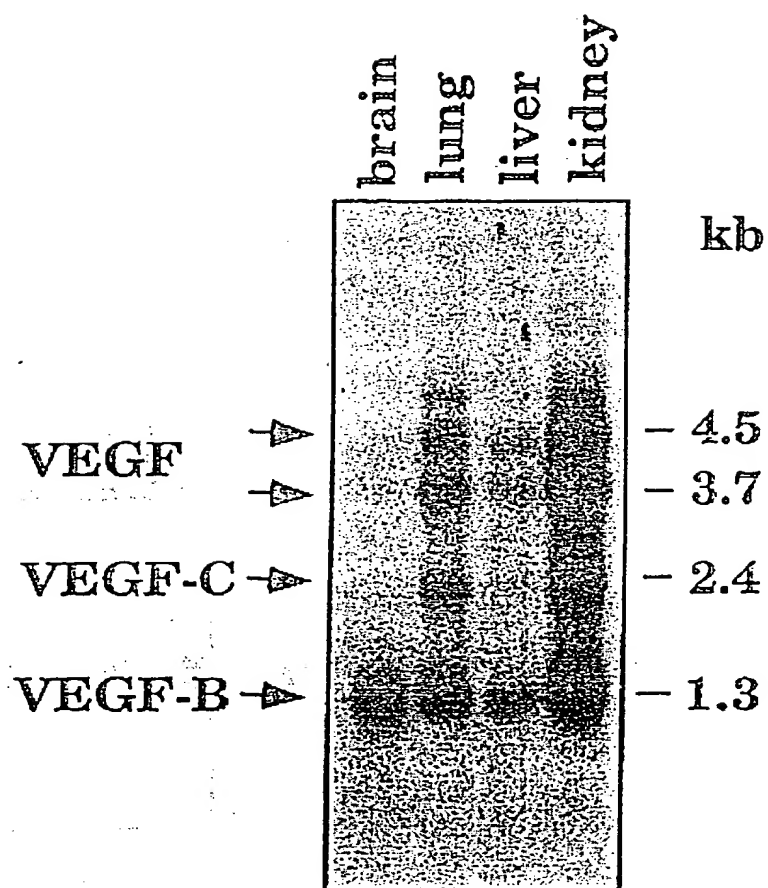


FIG. 16B

Chr 4



I VEGF-C,q34

FIG. 17

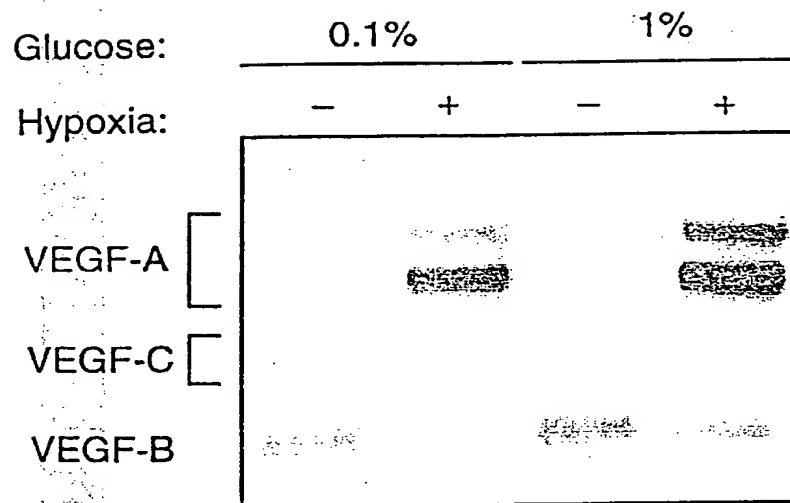


FIG. 18

OTHER PUBLICATIONS

- Cohen, T. et al., "VEGF₁₂₁, A Vascular Endothelial Growth Factor (VEGF) Isoform Lacking Heparin Binding Ability, Requires Cell-Surface Heparan Sulfates for Efficient Binding to the VEGF Receptors of Human Melanoma Cells" *Journal of Biological Chemistry*, 270(19):11322-11326 (May 12, 1995).
- Genbank AA151613, "z127h03.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 3'." Hillier, L. et al., Dated May 14, 1997.
- Genbank AA425486 "zw46b06.r1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 5' similar to SW:VEGF_MOUSE Q00731 Vascular Endothelial Growth Factor Precursor," Deposited by Hillier, L. et al. Dated Oct. 16, 1997.
- Genbank N31713, "yy15b12.s1 Homo sapiens cDNA clone 271295 3'." Deposited by Hillier, L. et al. Dated Jan. 10, 1996.
- Genbank N31720, "yy15d12.s1 Homo sapiens cDNA clone 271319 3'." Deposited by Hillier, L. et al. Dated Jan. 10, 1996.
- Genbank AA406492, "zv12g06.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 75366 5'." Deposited by Hillier, L. et al. Dated May 17, 1997.
- Genbank N50972, "yy94b08.s1 Homo sapiens cDNA clone 281175 3'." Deposited by Hillier, L. et al. Dated Feb. 14, 1996.
- Genbank AA421713, "zu24b03.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 738893 3'." Deposited by Hillier, L. et al. Dated Oct. 16, 1997.
- Genbank N94399, "zb76f04.s1 Soares senescent fibroblasts NbHSF Homo sapiens cDNA clone 309535 3'." Deposited by Hillier, L. et al. Dated Aug. 20, 1996.
- Genbank H05177, "y185b08.r1 Homo sapiens cDNA clone 44993 5'." Deposited by Hillier, L. et al. Dated Jun. 21, 1995.
- Genbank AA479987, "zv18h12.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 3'." Deposited by Hillier, L. et al. Dated Aug. 8, 1997.
- Genbank H05134, "y185b08.s1 Homo sapiens cDNA clone 44993 3'." Deposited by Hillier, L. et al. Dated Jun. 21, 1995.
- Genbank AA298182 "EST113866 Bone VII Homo sapiens cDNA 5' end." Deposited by Adams, M.D. et al. Dated Apr. 18, 1997.
- Genbank AA298283, "EST113896 Bone VII Homo sapiens cDNA 5' end similar to vascular endothelial growth factor." Deposited by Adams, M.D. et al. Dated Apr. 18, 1997.
- Genbank T81481, "yd29f07.s1 Homo sapiens cDNA clone 109669 3'." Deposited by Hillier, L. et al. Dated Mar. 15, 1995.
- Genbank AA425303, "zw46b06.r1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 3'." mRNA sequence." Deposited by Hillier, L. et al. Dated Oct. 16, 1997.
- Genbank Z40230, "H. sapiens partial cDNA sequence; clone c-1wf11." Deposited by Genexpress. Dated Sep. 21, 1995.
- Genbank Z44272, "H. sapiens partial cDNA sequence; clone c-1wf11." Deposited by Genexpress. Dated Sep. 21, 1995.
- Genbank AA478766, "zv18h12.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 5'." Deposited by Hillier, L. et al. Dated Aug. 8, 1997.
- Genbank H96876, "yw04b12.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 3'." Deposited by Hillier, L. et al. Dated Nov. 25, 1996.
- Genbank H96533, "yw04b12.r1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 5'." Deposited by Hillier, L. et al. Dated Nov. 25, 1996.
- Genbank T81690, "yd29f07.r1 Homo sapiens cDNA clone 109669 5' similar to SP:BAR3_CHITE Q03376 BALBI-ANI RING PROTEIN 3." Deposited by Hillier, L. et al. Dated Mar. 15, 1995.
- Genbank T84377, "yd37h08.r1 Homo sapiens cDNA clone 110463 5' similar to SP:BAR3_CHITE Q03376 BALBI-ANI RING PROTEIN 3." Deposited by Hillier, L. et al. Dated Mar. 16, 1995.
- Genbank N42368, "yy15b11.r1 Homo sapiens cDNA clone 271293 5'." Deposited by Hillier, L. et al. Dated Jan. 25, 1996.
- Genbank N42374, "yy15b11.r1 Homo sapiens cDNA clone 271317 5'." Deposited by Hillier, L. et al. Dated Jan. 25, 1996.
- Genbank H81868, "yv83d09.s1 Homo sapiens cDNA clone 249329 3'." Deposited by Hillier, L. et al. Dated Nov. 9, 1995.
- Genbank H81867, "yv83d09.r1 Homo sapiens cDNA clone 249329 5'." Deposited by Hillier, L. et al. Dated Nov. 9, 1995.
- Genbank AA149461, "z127h03.r1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 5' similar to SW:BAR3_CHITE Q03376 BALBI-ANI RING PROTEIN 3 PRECURSOR." Deposited by Hillier, L. et al. Dated May 14, 1997.
- Genbank R77495, "yi79e04.s1 Homo sapiens cDNA clone 145470 3'." Deposited by Hillier, L. et al. Dated Jun. 7, 1995.
- Genbank H07899, "y186g06.s1 Homo sapiens cDNA clone 45138 3'." Deposited by Hillier, L. et al. Dated Jun. 23, 1995.
- Genbank T89295, "yd37h08.s1 Homo sapiens cDNA clone 110463 3'." Deposited by Hillier, L. et al. Dated Mar. 20, 1995.
- Genbank C21512, "HUMGS0010510, Human Gene Signature, 3'-directed cDNA sequence." Deposited by Okubo, K. Dated Oct. 1, 1996.
- Genbank N82975, "TgESTzy53h10.r1 TgRH Tachyzoite cDNA Toxoplasma gondii cDNA clone tgy53h10.r1 5'." Deposited by Hehl, A. et al. Dated Sep. 10, 1997.
- Genbank AA285997, "vb88h06.r1 Soares mouse 3NbMS Mus musculus cDNA clone 764123 5'." Deposited by Marra, M. et al. Dated Apr. 9, 1997.
- Genbank AA549856, "0929m3 gmbPTHB3.1, G. Roman Reddy Plasmodium falciparum genomic clone 0929m." Deposited by Dame, J.B. et al. Dated Aug. 11, 1997.
- Jeltsch, M. et al., "Hyperplasia of Lymphatic Vessels in VEGF-C Transgenic Mice," *Science*, 276:1425-1425 (May, 1997).
- Joukov, V. et al., "Proteolytic Processing Regulates Receptor Specificity and Activity of VEGF-C," *EMBO Journal*, 16(13):3898-3911 (Jun., 1997).
- Joukov, V. et al., "A Recombinant Mutant Vascular Endothelial Growth Factor-C that has Lost Vascular Endothelial Growth Factor Receptor-2 Binding, Activation, and Vascular Permeability Activities," *Journal of Biological Chemistry*, 273(12):6599-6602 (Mar. 20, 1998).

- Lee, J. et al., "Vascular Endothelial Growth Factor Related Protein (vfp): A Ligand and Specific Activator of the Tyrosine Kinase Receptor Flt4," *EMBL Sequence Data Library*, XP002066361, accession No. U4142, Dated Jan. 10, 1996.
- Andersson et al., "Assignment of Interchain Disulfide Bonds in platelet-Derived Growth Factor (PDGF) and Evidence for Agonist Activity of Monomeric PDGF," *J. Biol. Chem.*, 267(16):11260-11266 (Jun. 5, 1992).
- Aprelikova et al., "FLT4, A Novel Class III Receptor Tyrosine Kinase in Chromosome 5q33-qter," *Cancer Research*, 52:746-748 (Feb. 1, 1992).
- Ausprunk, et al., "Migration and Proliferation of Endothelial Cells in Preformed and Newly Formed Blood Vessels during Tumor Angiogenesis," *Microvasc. Res.*, 14:53-65 (1977).
- Basilico et al., "The FGF Family of Growth Factors and Oncogenes," *Adv. Cancer Res.*, 59:145-165 (1992).
- Berse et al., "Vascular Permeability Factor (Vascular Endothelial Growth Factor) Gene is Expressed Differentially in Normal Tissues, Macrophages, and Tumors," *Mol. Biol. Cell.*, 3:211-220 (Feb., 1992).
- Beisholtz et al., "cDNA Sequence and Chromosomal Localization of Human Platelet-Derived Growth Factor A-Chain and Its Expression in Tumor Cell Lines," *Nature*, 320:695-699 (Apr., 1986).
- Borg et al., "Biochemical Characterization of Two Isoforms of FLT4, a VEGF Receptor-Related Tyrosine Kinase," *Oncogene*, 10:973-84 (1995).
- Breier et al., "Expression of Vascular Endothelial Growth Factor During Embryonic Angiogenesis and Endothelial Cell Differentiation," *Development*, 114:521-532 (1992).
- Cao et al., "Heterodimers of Placenta Growth Factor/Vascular Endothelial Growth Factor," *J. Biol. Chem.*, 271(6):3154-3162 (Feb. 9, 1996).
- Cheng and Flanagan, "Identification and Cloning of ELF-1, A Developmentally Expressed Ligand for the Mek4 and Sek Receptor Tyrosine Kinases," *Cell*, 79:157-168 (Oct. 7, 1994).
- Claesson-Welsh et al., "Identification and Structural Analysis of the A Type Receptor for Platelet-derived Growth Factor," *J. Biol. Chem.*, 264(3):1742-1747 (Jan. 25, 1989).
- Coffin et al., "Angioblast Differentiation and Morphogenesis of the Vascular Endothelium in the Mouse Embryo," *Devel. Biol.*, 148:51-62 (1991).
- Curran and Franza, "Fos and Jun: The AP-1 Connection," *Cell*, 55:395-397 (Nov. 4, 1988).
- De Vries et al., "The fms-Like Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor," *Science*, 255:989-991 (Feb. 21, 1992).
- Dignam et al., "Balbiani Ring 3 in *Chironomus tentans* Encodes a 185-kDa Secretory Protein Which is Synthesized Throughout the Fourth Larval Instar," *Gene*, 88:133-140 (1990).
- DiSalvo et al., "Purification and Characterization of a Naturally Occurring Vascular Endothelial Growth Factor: Placenta Growth Factor Heterodimer," *J. Biol. Chem.*, 270(13):7717-7723 (Mar. 31, 1995).
- Don et al., "'Touchdown' PCR to Circumvent Spurious Priming During Gene Amplification," *Nucl. Acids Res.*, 19:4008 (1991).
- Dumont et al., "Dominant-negative and Targeted Null Mutations in the Endothelial Receptor Tyrosine Kinase, tek, Reveal a Critical Role in Vasculogenesis of the Embryo," *Genes Dev.*, 8:1897-1909 (1994).
- Dumont et al., "Vascularization of the Mouse Embryo: A Study of flk-1, tek, tie and Vascular Endothelial Growth Factor Expression During Development," *Development Dynamics*, 203:80-92 (1995).
- Dvorak et al., "Review: Vascular Permeability Factor/Vascular Endothelial Growth Factor, Microvascular Hyperpermeability, and Angiogenesis," *Amer. J. Path.*, 146:1029-1039 (1995).
- Eichmann et al., "Two Molecules Related to the VEGF Receptor are Expressed in Early Endothelial Cells During Avian Embryonic Development," *Mech. Dev.*, 42:33-48 (1993).
- Ferrara et al., "Molecular and Biological Properties of the Vascular Endothelial Growth Factor Family of Proteins," *Endocrine Rev.*, 13(1):18-32 (1992).
- Finnerty et al., "Molecular Cloning of Murine FLT and FLT4," *Oncogene*, 8(11):2293-2298 (1993).
- Fiamme et al., "Vascular Endothelial Growth Factor (VEGF) and VEGF-Receptor 2 (flk-1) are Expressed During Vasculogenesis and Vascular Differentiation in the Quail Embryo," *Devel. Biol.*, 169:699-712 (1995).
- Flanagan and Leder, "The kit Ligand: A Cell Surface Molecule Altered in Steel Mutant Fibroblasts," *Cell*, 63:185-194 (Oct. 5, 1990).
- Folkman, "Angiogenesis in Cancer, Vascular, Rheumatoid and Other Disease," *Nature Med.*, 1(1):27-31 (1995).
- Folkman et al., "Angiogenesis," *J. Biol. Chem.*, 267(16):10931-10934 (Jun. 5, 1992).
- Folkman et al., "Long-term Culture of Capillary Endothelial Cells," *Proc. Nat'l Acad. Sci., USA*, 76(10):5217-5221 (Oct., 1979).
- Fong et al., "Role of the Flt-1 Receptor Tyrosine Kinase in Regulating the Assembly of Vascular Endothelium," *Nature*, 376:66-70 (Jul. 6, 1995).
- Fournier et al., "Mutation of Tyrosine Residue 1337 Abrogates Ligand-Dependent Transforming Capacity of the FLT4 Receptor," *Oncogene*, 11(5):921-931 (Sep. 7, 1995).
- Friesel et al., "Molecular Mechanisms of Angiogenesis: Fibroblast Growth Factor Signal Transduction," *FASEB J.*, 9:919-25 (1995).
- Galland et al., "The Flt4 Gene Encodes a Transmembrane Tyrosine Kinase Related to the Vascular Endothelial Growth Factor Receptor," *Oncogene*, 8:1233-1240 (1993).
- Genbank S66407, "FLT4 Receptor Tyrosine Kinase Isoform FLT4 Long (3' Region, Alternatively Spliced) [Human, mRNA Partial, 216 nt]," Deposited by Pajusola et al., Dated Dec. 17, 1993.
- Genbank U48800, "Mus Musculus Vascular Endothelial Growth Factor B Precursor (VEGF-B) mRNA, Complete Cds.," Deposited by Olofsson et al., Dated Aug. 19, 1996.
- Genbank X15997, "Human Vascular Permeability Factor mRNA, Complete Cds.," Deposited by Keck et al., Dated Jun. 15, 1990.
- Genbank X60280, "Vector Plasmid pLTRpoly DNA.," Deposited by Maekelae, T.P., Dated Jul. 16, 1996.
- Genbank X68203, "*H. sapiens* mRNA for FLT4, Class III Receptor Tyrosine Kinase.," Deposited by Aprelikova, O., Dated Nov. 30, 1993.
- Genbank X94216, "*Homo sapiens* mRNA for VEGF-C protein," Deposited by Joukov et al., Dated Feb. 6, 1996.
- Harlow et al., *Antibodies, a Laboratory Manual*, Cold Spring Harbor Laboratory Press, pp. 72-137, 141-157, 287 & 321-358 (1988).

- Heldin et al., "Structure of Platelet-Derived Growth Factor: Implications for Functional Properties," *Growth Factors*, 8:245-252 (1993).
- Joukov et al., "A Novel Vascular Endothelial Growth Factor, VEGF-C, Is a Ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) Receptor Tyrosine Kinases," *EMBO J.*, 15(2):290-298 (1996).
- Kaipainen et al., "Expression of the FMS-Like Tyrosine Kinase 4 Gene Becomes Restricted to Lymphatic Endothelium During Development," *Proc. Nat'l Acad. Sci., USA*, 92:3566-3570 (Apr., 1995).
- Kaipainen et al., "The Related FLT4, FLT1 and KDR Receptor Tyrosine Kinases Show Distinct Expression Patterns in Human Fetal Endothelial Cells," *J. Exp. Med.*, 178:2077-2088 (Dec., 1993).
- Kaipainen et al., "Enhanced Expression of the Tie Receptor Tyrosine Kinase Messenger RNA in the Vascular Endothelium of Metastatic Melanomas," *Cancer Res.*, 54:6571-6577 (Dec. 15, 1994).
- Kozak, "An Analysis of 5'-Non-Coding Sequences from 699 Vertebrate Messenger RNAs," *Nucl. Acids Res.*, 15:8125-8148 (1987).
- Lee et al., "Vascular Endothelial Growth Factor-Related Protein: A Ligand and Specific Activator of the Tyrosine Kinase Receptor Flt4," *Proc. Nat'l Acad. Sci., USA*, 93:1988-1992 (Mar., 1996).
- Leung et al., "Vascular Endothelial Growth Factor Is a Secreted Angiogenic Mitogen," *Science*, 246:1306-1309 (Dec. 8, 1989).
- Levy et al., "Post-transcriptional Regulation of Vascular Endothelial Growth Factor by Hypoxia," *J. Biol. Chem.*, 271(5):2746-2753 (Feb. 2, 1996).
- Levy et al., "Transcriptional Regulation of the Rat Vascular Endothelial Growth Factor Gene by Hypoxia," *J. Biol. Chem.*, 270(22):13333-13340 (Jun. 2, 1995).
- Lyman et al., "Molecular Cloning of a Ligand for the flt3/flk-2 Tyrosine Kinase Receptor: A Proliferative Factor for Primitive Hematopoietic Cells," *Cell*, 75:1157-1167 (Dec. 17, 1993).
- Maglione et al., "Isolation of a Human Placenta cDNA Coding for a Protein Related to the Vascular Permeability Factor," *Proc. Nat'l Acad. Sci., USA*, 88:9267-9271 (Oct., 1991).
- Maglione et al., "Two Alternative mRNAs Coding for the Angiogenic Factor, Placenta Growth Factor (PlGF) are Transcribed from a Single Gene of Chromosome 14," *Oncogene*, 8:925-931 (1993).
- Mäkelä et al., "Plasmid pLTRpoly: A Versatile High-Efficiency Mammalian Expression Vector," *Gene*, 118: 293-294 (1992).
- Matthews et al., "A Receptor Tyrosine Kinase cDNA Isolated from a Population of Enriched Primitive Hematopoietic Cells and Exhibiting Close Genetic Linkage to c-kit," *Proc. Nat'l Acad. Sci., USA*, 88:9026-9030 (Oct., 1991).
- Meitzelaar et al., "CD63 Antigen," *J. of Biol. Chem.*, 266(5):3239-3245 (Feb. 15, 1991).
- Millauer et al., "Glioblastoma Growth Inhibited in vivo by a Dominant-Negative Flk-1 Mutant," *Nature*, 367:576-579 (Feb. 10, 1994).
- Millauer et al., "High Affinity VEGF Binding and Developmental Expression Suggest Flk-1 as a Major Regulator of Vasculogenesis and Angiogenesis," *Cell*, 72:835-846 (Mar. 26, 1993).
- Mitchell et al., "Transcription Factor AP-2 is Expressed in Neural Crest Cell Lineages During Mouse Embryogenesis," *Genes and Dev.*, 5:105-119 (1991).
- Morgenstern et al., "Advanced Mammalian Gene Transfer: High Titer Retroviral Vectors With Multiple Drug Selection Markers and a Complementary Helper-Free Packaging Cell Line," *Nucl. Acids Res.*, 18(12):3587-3595 (1990).
- Mount, S.M., "A Catalogue of Splice Junction Sequences," *Nucl. Acids Res.*, 10(2):459-472 (1982).
- Mustonen et al., "Endothelial Receptor Tyrosine Kinases Involved in Angiogenesis," *J. Cell Biol.*, 129:895-898 (May, 1995).
- Nelson and Sun, "The 50- and 58-kdalton Keratin Classes as Molecular Markers for Stratified Squamous Epithelia: Cell Culture Studies," *J. Cell Biol.*, 97:244-251 (Jul., 1983).
- Neufeld et al., "Vascular Endothelial Growth Factor and Its Receptors," *Prog. Growth Fact. Res.*, 5:89-97 (1994).
- Oefner et al., "Crystal Structure of Human Platelet-derived Growth Factor BB," *EMBO J.*, 11(11):3921-3926 (1992).
- Oelrichs et al., "NYK/FLK-1: A Putative Receptor Tyrosine Kinase Isolated from E10 Embryonic Neuroepithelium is Expressed in Endothelial Cells of the Developing Embryo," *Oncogene*, 8:11-18 (1993).
- Olofsson et al., "Vascular Endothelial Growth Factor B, A Novel Growth Factor for Endothelial Cells," *Proc. Nat'l Acad. Sci., USA*, 93:2576-2581 (Mar., 1996).
- Paavonen et al., "Novel Human Vascular Endothelial Growth Factor Genes VEGF-B and VEGF-C Localize to Chromosomes 11q13 and 4q34, Respectively," *Circulation*, 93(6):1079-1082 (Mar. 15, 1996).
- Pajusola et al., "FLT4 Receptor Tyrosine Kinase Contains Seven Immunoglobulin-Like Loops and Is Expressed in Multiple Human Tissues and Cell Lines," *Cancer Res.*, 52:5738-5743 (Oct. 15, 1992).
- Pajusola et al., "Signaling Properties of FLT4, a Proteolytically Processed Receptor Tyrosine Kinase Related to Two VEGF Receptors," *Oncogene*, 9:3545-3555 (1994).
- Pajusola et al., "Two Human FLT4 Receptor Tyrosine Kinase Isoforms With Distinct Carboxy Terminal Tails are Produced by Alternative Processing of Primary Transcripts," *Oncogene*, 8: 2931-2937 (1993).
- Park et al., "Placenta Growth Factor, Potentiation of Vascular Endothelial Growth Factor Bioactivity In vitro and In vivo, and High Affinity Binding to Flt-1 but not to Flk-1/KDR," *J. Biol. Chem.*, 269(41):25646-25654 (Oct. 14, 1994).
- Partanen et al., "A Novel Endothelial Cell Surface Receptor Tyrosine Kinase with Extracellular Epidermal Growth Factor Homology Domains," *Mol. & Cell. Biol.*, 12(4):1698-1707 (Apr., 1992).
- Partanen et al., "Putative Tyrosine Kinases Expressed in K-562 Human Leukemia Cells," *Proc. Nat'l Acad. Sci., USA*, 87:5715-5717 (Nov., 1990).
- Paulsson et al., "The Balbiani Ring 3 Gene in *Chironomus tentans* has a Diverged Repetitive Structure Split by Many Introns," *J. Mol. Biol.*, 211:331-349 (1990).
- Pear et al., "Production of High-titer Helper-free Retroviruses by Transient Transfection," *Proc. Nat'l Acad. Sci., USA*, 90:8392-8396 (Sep., 1993).
- Pertovaara et al., "Vascular Endothelial Growth Factor Is Induced in Response to Transforming Growth Factor- β in Fibroblastic and Epithelial Cells," *J. Biol. Chem.*, 269(9):6271-6274 (Mar. 4, 1994).

- Peters et al., "Vascular Endothelial Growth Factor Receptor Expression during Embryogenesis and Tissue Repair Suggests a Role in Endothelial Differentiation and Blood Vessel Growth," *Proc. Nat'l Acad. Sci., USA*, 90:8915-8918 (Oct., 1993).
- Pötgens et al., "Covalent Dimerization of Vascular Permeability Factor/Vascular Endothelial Growth Factor Is Essential for Its Biological Activity," *J. Biol. Chem.*, 269(52):32879-32885 (Dec. 30, 1994).
- Puri et al., "The Receptor Tyrosine Kinase TIE is Required for Integrity and Survival of Vascular Endothelial Cells," *EMBO J.*, 14:5884-5891 (1995).
- Quinn et al., "Fetal Liver Kinase I is a Receptor for Vascular Endothelial Growth Factor and is Selectively Expressed in Vascular Endothelium," *Proc. Nat'l Acad. Sci., USA*, 90:7533-7537 (Aug., 1993).
- Risau et al., "Changes on the Vascular Extracellular Matrix During Embryonic Vasculogenesis and Angiogenesis," *Devel. Biol.*, 125:441-450 (1988).
- Risau et al., "Platelet-Derived Growth Factor is Angiogenic In Vivo," *Growth Factors*, 7:261-266 (1992).
- Risau, W., "Differentiation of Endothelium," *FASEB J.*, 9:926-933 (1995).
- Sabin, F.R., "The Lymphatic System in Human Embryos, With A Consideration of the Morphology of the System as a Whole," *Am. J. Anat.*, 9(1):43-91 (1909).
- Saksela et al., "Cell-Associated Plasminogen Activation: Regulation and Physiological Function," *Ann. Rev. Cell Biol.*, 4:93-126 (1988).
- Sambrook et al., *Molecular Cloning: a Laboratory Manual*, Second Edition, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory (1989), pp. 2.60-2.79, 4.21-4.32, 7.3-7.36, and 9.47-9.51.
- Sato et al., "Distinct Roles of the Receptor Tyrosine Kinases Tie-1 and Tie-2 in Blood Vessel Formation," *Nature*, 376:70-74 (Jul. 6, 1995).
- Schneider et al., "A One-step Purification of Membrane Proteins Using a High Efficiency Immunomatrix," *J. Biol. Chem.*, 257(18):10766-10769 (Sep. 25, 1982).
- Seetharam et al., "A Unique Signal Transduction from FLT Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor VEGF," *Oncogene*, 10:135-147 (1995).
- Senger et al., "Tumor Cells Secrete a Vascular Permeability Factor That Promotes Accumulation of Ascites Fluid," *Science*, 219:983-985 (Feb. 25, 1983).
- Shalaby et al., "Failure of Blood-Island Formation and Vasculogenesis in Flk-1-deficient Mice," *Nature*, 376:62-66 (Jul. 6, 1995).
- Shibuya et al., "Nucleotide Sequence and Expression of a Novel Human Receptor-Type Tyrosine Kinase Gene (flt) Closely Related to the fms Family," *Oncogene*, 5:519-524 (1990).
- Shibuya, M., "Role of VEGF-FLT Receptor System in Normal and Tumor Angiogenesis," *Adv. Cancer Res.*, 67:281-316 (1995).
- Shweiki et al., "Patterns of Expression of Vascular Endothelial Growth Factor (VEGF) and VEGF Receptors in Mice Suggest a Role in Hormonally Regulated Angiogenesis," *J. Clin. Invest.*, 91:2235-2243 (May, 1993).
- Sitaras et al., "Constitutive Production of Platelet-Derived Growth Factor-Like Proteins by Human Prostate Carcinoma Cell Lines," *Cancer Research*, 48(7):1930-1935 (Apr. 1, 1988).
- Southern and Berg, "Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter," *J. Mol. Appl. Genet.*, 1:327-341 (1982).
- Terman et al., "Identification of New Endothelial Cell Growth Factor Receptor Tyrosine Kinase," *Oncogene*, 6:1677-1683 (1991).
- Terman et al., "Identification of the KDR Tyrosine Kinase as a Receptor for Vascular Endothelial Cell Growth Factor," *Biochem. Biophys. Res. Commun.*, 187:1579-1586 (Sep. 30, 1992).
- Terman et al., "VEGF Receptor Subtypes KDR and FLT1 Show Different Sensitivities to Heparin and Placenta Growth Factor," *Growth Factors*, 11(3):187-195 (1994).
- Tessier et al., "Enhanced Secretion From Insect Cells of a Foreign Protein Fused to the Honeybee Melittin Signal Peptide," *Gene*, 98: 177-183 (1991).
- Tischer et al., "The Human Gene for Vascular Endothelial Growth Factor, Multiple Protein Forms are Encoded Through Alternative Exon Splicing," *J. Biol. Chem.*, 266(18):11947-11954 (Jun. 25, 1991).
- Van der Geer et al., "Receptor Protein-Tyrosine Kinases and Their Signal Transduction Pathways," *Ann. Rev. Cell Biol.*, 10:251-337 (1994).
- Vassar et al., "Tissue-specific and Differentiation-specific Expression of a Human K14 Keratin Gene in Transgenic Mice," *Proc. Nat'l Acad. Sci., USA*, 86:1563-1567 (Mar., 1989).
- Vassar et al., "Transgenic Mice Provide New Insights Into the Role of TGF- α During Epidermal Development and Differentiation," *Genes & Dev.*, 5:714-727 (1991).
- Västrik et al., "Expression of the Mad Gene During Cell Differentiation In Vivo and Its Inhibition of Cell Growth In Vitro," *J. Cell. Biol.*, 128(6):1197-1208 (Mar., 1995).
- von Heijne, G., "A New Method for Predicting Signal Sequence Cleavage Sites," *Nucleic Acids Res.*, 14(11):4683-4690 (1986).
- Waltenberger et al., "Different Signal Transduction Properties of KDR and Flt1, Two Receptors for Vascular Endothelial Growth Factor," *J. Biol. Chem.*, 269(43):26988-26995 (Oct. 28, 1994).
- Wanaka et al., "Expression of FGF Receptor Gene in Rat Development," *Development*, 111:455-468 (1991).
- Wen et al., "Neu Differentiation Factor: A Transmembrane Glycoprotein Containing an EGF Domain and an Immunoglobulin Homology Unit," *Cell* 69:559-572 (May 1, 1992).
- Yamane et al., "A New Communication System Between Hepatocytes and Sinusoidal Endothelial Cells in Liver Through Vascular Endothelial Growth Factor and Flt Tyrosine Kinase Receptor Family (Flt-1 and KDR/Flk-1)," *Oncogene*, 9:2683-2690 (1994).

* cited by examiner

RECEPTOR LIGAND

This is a continuation-in-part of U.S. patent application Ser. No. 08/510,133, filed Aug. 1, 1995.

FIELD OF THE INVENTION

The present invention generally relates to the field of genetic engineering and more particularly to growth factors for endothelial cells and growth factor genes.

BACKGROUND OF THE INVENTION

Developmental growth, the remodelling and regeneration of adult tissues, as well as solid tumor growth, can only occur when accompanied by blood vessel formation. Angioblasts and hematopoietic precursor cells differentiate from the mesoderm and form the blood islands of the yolk sac and the primary vascular system of the embryo. The development of blood vessels from these early (in situ) differentiating endothelial cells is termed vasculogenesis. Major embryonic blood vessels are believed to arise via vasculogenesis, whereas the formation of the rest of the vascular tree is thought to occur as a result of vascular sprouting from pre-existing vessels, a process called angiogenesis. Risau, et al., *Devel. Biol.*, 125:441-450 (1988).

Endothelial cells give rise to several types of functionally and morphologically distinct vessels. When organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases. Upon angiogenic stimulation, endothelial cells may re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their tissue environment. Endothelial cells undergoing angiogenesis degrade the underlying basement membrane and migrate, forming capillary sprouts that project into the perivascular stroma. Ausprunk, et al., *Microvasc. Rev.*, 14:51-65 (1977). Angiogenesis during tissue development and regeneration depends on the tightly controlled processes of endothelial cell proliferation, migration, differentiation, and survival. Dysfunction of the endothelial cell regulatory system is a key feature of many diseases. Most significantly, tumor growth and metastasis have been shown to be angiogenesis dependent. Folkman, et al., *J. Biol. Chem.*, 267: 10931-10934 (1992).

Key signals regulating cell growth and differentiation are mediated by polypeptide growth factors and their transmembrane receptors, many of which are tyrosine kinases. Autophosphorylated peptides within the tyrosine kinase insert and carboxyl-terminal sequences of activated receptors are commonly recognized by kinase substrates involved in signal transduction for the readjustment of gene expression in responding cells. Several families of receptor tyrosine kinases have been characterized. Van der Geer, et al., *Ann. Rev. Cell Biol.*, 10:251-337 (1994). The major growth factors and receptors transducing angiogenic stimuli are schematically shown in FIG. 1.

Fibroblast growth factors are also known to be involved in the regulation of angiogenesis. They have been shown to be mitogenic and chemotactic for cultured endothelial cells. Fibroblast growth factors also stimulate the production of proteases, such as collagenases and plasminogen activators, and induce tube formation by endothelial cells. Saksela, et al., *Ann. Rev. Cell Biol.*, 4:93-126 (1988). There are two general classes of fibroblast growth factors, FGF-1 and FGF-2, both of which lack conventional signal peptides. Both types have an affinity for heparin and FGF-2 is bound

to heparin sulfate proteoglycans in the subendothelial extracellular matrix from which it may be released after injury. Heparin potentiates the stimulation of endothelial cell proliferation by angiogenic FGFs, both by protecting against denaturation and degradation and dimerizing the FGFs. Cultured endothelial cells express the FGF-1 receptor but no significant levels of other high-affinity fibroblast growth factor receptors.

Among other ligands for receptor tyrosine kinases, the platelet derived growth factor, PDGF-BB, has been shown to be weakly angiogenic in the chick chorioallantoic membrane. Risau, et al., *Growth Factors*, 7:261-266 (1992). Transforming growth factor α (TGF α) is an angiogenic factor secreted by several tumor cell types and by macrophages. Hepatocyte growth factor (HGF), the ligand of the c-met proto-oncogene-encoded receptor, also is strongly angiogenic.

Recent evidence shows that there are endothelial cell specific growth factors and receptors that may be primarily responsible for the stimulation of endothelial cell growth, differentiation and certain differentiated functions. The best studied of these is vascular endothelial growth factor (VEGF), a member of the PDGF family. Vascular endothelial growth factor is a dimeric glycoprotein of disulfide-linked 23 kDa subunits. Other reported effects of VEGF include the mobilization of intracellular calcium, the induction of plasminogen activator and plasminogen activator inhibitor-1 synthesis, stimulation of hexose transport in endothelial cells, and promotion of monocyte migration in vitro. Four VEGF isoforms, encoded by distinct mRNA splice variants, appear to be equally capable of stimulating mitogenesis in endothelial cells. However, each isoform has a different affinity for cell surface proteoglycans, which behave as low affinity receptors for VEGF. The 121 and 165 amino acid isoforms of VEGF are secreted in a soluble form, whereas the isoforms of 189 and 206 amino acid residues remain cell surface associated and have a strong affinity for heparin.

VEGF was originally purified from several sources on the basis of its mitogenic activity toward endothelial cells, and also by its ability to induce microvascular permeability, hence it is also called vascular permeability factor (VPF). VEGF produces signals through two receptor tyrosine kinases, VEGFR-1 (FLT-1) and VEGFR-2 (KDR/Flk-1), which are expressed specifically on endothelial cells. The VEGF-related placenta growth factor (PlGF) was recently shown to bind to VEGFR-1 with high affinity. PlGF was able to enhance the growth factor activity of VEGF, but it did not stimulate endothelial cells on its own. Naturally occurring VEGF/PlGF heterodimers were nearly as potent mitogens as VEGF homodimers for endothelial cells.

The pattern of VEGF expression suggests its involvement in the development and maintenance of the normal vascular system and in tumor angiogenesis. During murine development, the entire 7.5 day post-coital (p.c.) endoderm expresses VEGF and the ventral midline mesoderm produces VEGF at the capillary ingrowth stage. See Breier, et al., *Development*, 114:521-523 (1992). On day two of quail development, the vascularized area of the yolk sac as well as the whole embryo show expression of VEGF. In addition, epithelial cells next to fenestrated endothelia in adult mice show persistent VEGF expression, suggesting a role in the maintenance of this specific endothelial phenotype and function.

Two high affinity receptors for VEGF have been characterized. These are VEGFR-1/Flt-1 (fms-like tyrosine kinase-

1) and VEGFR-2/Kdr/Flk-1 (kinase insert domain containing receptor/fetal liver kinase-1). Those receptors are classified in the PDGF-receptor family, but they have seven rather than five immunoglobulin-like loops in their extracellular domain and they possess a longer kinase insert than normally observed in this family. The expression of VEGF receptors occurs mainly in vascular endothelial cells, although some may be present on monocytes and melanoma cells. Only endothelial cells have been reported to proliferate in response to VEGF, and endothelial cells from different sources show different responses. Thus, the signals mediated through VEGFR-1 and VEGFR-2 appear to be cell type specific.

The Flt4 receptor tyrosine kinase (VEGFR-3) is closely related in structure to the products of the VEGFR-1 and VEGFR-2 genes. Despite this similarity, the mature form of Flt4 differs from the VEGF receptors in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola et al., *Cancer Res.*, 52:5738-5743 (1992). The 4.5 and 5.8 kb Flt4 mRNAs encode polypeptides which differ in their C-termini due to the use of alternative 3' exons. The VEGFs do not show specific binding to Flt4 or induce its autophosphorylation.

Expression of Flt4 appears to be more restricted than expression of VEGFR-1 or VEGFR-2. The expression of Flt4 first becomes detectable by in situ hybridization in the angioblasts of head mesenchyme, the cardinal vein, and extraembryonically in the allantois of 8.5 day p.c. mouse embryos. In 12.5 day p.c. embryos the Flt4 signal is observed in developing venous and presumptive lymphatic endothelia, but arterial endothelia appear negative. During later stages of development, Flt4 mRNA becomes restricted to developing lymphatic vessels. Only the lymphatic endothelia and some high endothelial venules express Flt4 mRNA in adult human tissues and increased expression occurs in lymphatic sinuses in metastatic lymph nodes and in lymphangioma. These results support the theory of the venous origin of lymphatic vessels.

Five endothelial cell-specific receptor tyrosine kinases, Flt-1 (VEGFR-1), KDR/Flk-1 (VEGFR-2), Flt4, Tie and Tek/Tie-2 have so far been described, which possess the intrinsic tyrosine kinase activity essential for signal transduction. Targeted mutations inactivating Flt-1, Flk-1, Tie and Tek in mouse embryos have indicated their essential and specific roles in vasculogenesis and angiogenesis at the molecular level. VEGFR-1 and VEGFR-2 bind VEGF with high affinity (Kd 16 pM and 760 pM, respectively) and VEGFR-1 also binds the related placenta growth factor (PIGF; Kd about 200 pM), while the ligands for Tie, Tek, and Flt4 have not yet been reported.

SUMMARY OF THE INVENTION

The present invention provides a ligand for the Flt4 receptor tyrosine kinase. Thus, the invention provides a purified and isolated polypeptide which specifically binds to the Flt4 receptor tyrosine kinase. In a preferred embodiment, the ligand comprises a fragment of the amino acid sequence shown in SEQ ID NO: 33 which specifically binds to the Flt4 receptor tyrosine kinase.

The present invention also provides a precursor of an Flt4 ligand, wherein the precursor comprises the amino acid sequence shown in SEQ ID NO: 33. Thus, the invention includes a purified and isolated polypeptide having the amino acid sequence shown in SEQ ID NO: 33.

A putative 33 amino acid signal peptide has been identified in the amino acid sequence shown in SEQ ID NO: 33.

Thus, in a related aspect, the invention includes a purified and isolated polypeptide comprising amino acids 1-317 of SEQ ID NO: 33. The Flt4 ligand precursor is proteolytically cleaved upon expression to produce an approximately 23 kD peptide which is the Flt4 ligand (herein designated VEGF-C). Thus, the invention includes a polypeptide having an amino acid sequence comprising a portion of SEQ ID NO: 33, the portion encoding a fragment capable of specifically binding to Flt4. A preferred fragment has a molecular weight of about 23 kDa as assessed by SDS-PAGE under reducing conditions. In a preferred embodiment of the invention, an Flt4 ligand is provided which is the cleavage product of the precursor peptide shown in SEQ ID NO: 33 and which has a molecular weight of approximately 23 kD under reducing conditions.

Evidence suggests that the amino acids essential for retaining Flt4 ligand activity are contained within approximately amino acids 1-120 of SEQ ID NO: 33, and that the proteolytic cleavage to produce a mature, naturally-occurring Flt4 ligand occurs within approximately amino acids 1-180 of SEQ ID NO: 33. Accordingly, preferred polypeptides of the invention include polypeptides comprising amino acids 1-120, 1-121, 1-122, 1-123, 1-124, 1-178, 1-179, and 1-180 of SEQ ID NO: 33, wherein said polypeptides specifically bind to an Flt4 receptor tyrosine kinase. A preferred Flt4 ligand comprises approximately amino acids 1-120 of SEQ ID NO: 33. Another preferred polypeptide of the invention comprises approximately amino acids 1-180 of SEQ ID NO: 33.

The present invention also provides a cDNA encoding a novel polypeptide, designated VEGF-C, that is structurally homologous to VEGF. VEGF-C is a ligand for the Flt4 receptor tyrosine kinase (VEGFR-3), a receptor tyrosine kinase related to VEGFR-1 and VEGFR-2 that does not bind VEGF. VEGFR-3 is expressed in venous and lymphatic endothelia of fetal tissues and predominantly in lymphatic endothelial of adult tissues. Kaipainen et al., *Cancer Res.*, 54:6571-77 (1994); Kaipainen et al., *Proc. Natl. Acad. Sci. USA*, 92:3566-70 (1995).

Thus, in a preferred embodiment, the invention includes a purified and isolated nucleic acid (e.g., a DNA or an RNA) encoding an Flt4 ligand precursor. Due to the degeneracy of the genetic code, numerous such coding sequences are possible, each having in common the coding of the amino acid sequence shown in SEQ ID NO: 33. As set forth above, the invention includes polypeptides which comprise a portion of the amino acid sequence shown in SEQ ID NO: 33 and which bind the Flt4 receptor tyrosine kinase (herein designated VEGFR-3); the invention also is intended to include nucleic acids encoding these polypeptides. Ligand precursors according to the invention, when expressed in an appropriate host cell, produce, via cleavage, a peptide which binds specifically to the Flt4 receptor tyrosine kinase (VEGFR-3). The nucleotide sequence shown in SEQ ID NO: 33 contains a preferred nucleotide sequence encoding the Flt4 ligand (VEGF-C).

The present invention also provides a cell line which produces an Flt4 ligand. The ligand may be purified and isolated directly from the cell culture medium. Also provided are vectors comprising a DNA encoding the Flt4 ligand, and host cells comprising the vectors. Preferred vectors of the invention are capable of expressing the Flt4 ligand under the control of appropriate promoters and other control sequences. A preferred vector of the invention is plasmid pFLT4-L, having ATCC accession no. 97231.

The invention further includes a method of making polypeptides of the invention. In a preferred method, a

nucleic acid or vector of the invention is expressed in a host cell, and a polypeptide of the invention is purified from the host cell or the host cell growth medium.

In another aspect, the invention includes an antibody which is specifically reactive with polypeptides of the invention. Antibodies, both monoclonal and polyclonal, may be made against a ligand of the invention according to standard techniques in the art. Such antibodies may be used in diagnostic applications to monitor angiogenesis, vascularization, lymphatic vessels and their disease states, wound healing, or certain hematopoietic or leukemia cells, or they may be used to block or activate the Flt4 receptor.

Ligands according to the invention may be labeled with a detectable label and used to identify their corresponding receptors *in situ*. Labeled Flt4 ligand and anti-Flt4 ligand antibodies may be used as imaging agents in the detection of lymphatic vessels, high endothelial venules, and Flt4 receptors expressed in histochemical tissue sections. The ligand or antibody may be covalently or non-covalently coupled to a suitable supermagnetic, paramagnetic, electron dense, echogenic, or radioactive agent for imaging. Other, non-radioactive labels, such as biotin and avidin, may also be used.

The present invention also provides diagnostic and clinical applications for claimed ligands. In a preferred embodiment, Flt4 ligands or precursors are used to accelerate angiogenesis, e.g., during wound healing, or to promote the endothelial functions of lymphatic vessels. Ligands may be applied in any suitable manner using an appropriate pharmaceutically-acceptable vehicle. Ligands also may be used to quantify future metastatic risk by assaying biopsy material for the presence of active receptors or ligands in a binding assay or kit using detectably-labeled ligand. An Flt4 ligand according to the invention also may be used to promote re-growth or permeability of lymphatic vessels in, for example, organ transplant patients. Ligands according to the invention also may be used to treat or prevent inflammation, edema, aplasia of the lymphatic vessels, lymphatic obstruction, elephantiasis, and Milroy's disease. Finally, Flt4 ligands may be used to stimulate lymphocyte production and maturation, and to promote or inhibit trafficking of leukocytes between tissues and lymphatic vessels or to affect migration in and out of the thymus.

Inhibitors of the Flt4 ligand may be used to control endothelial cell proliferation and lymphangiomas. For example, such inhibitors may be used to arrest metastatic growth or spread, or to control other aspects of endothelial cell expression and growth. Inhibitors include antibodies, antisense oligonucleotides, and peptides which block the Flt4 receptor, all of which are intended as aspects of the invention.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a schematic diagram showing major endothelial cell receptor tyrosine kinases and growth factors involved in vasculogenesis and angiogenesis.

FIGS. 2A and 2B schematically depict the construction of the pLTRFlt4l expression vector.

FIG. 3 schematically depicts the construction of the baculovirus vector encoding a secreted soluble Flt4 extracellular domain (Flt4EC).

FIG. 4 shows results of stimulation of Flt4 autophosphorylation by conditioned medium from PC-3 cell cultures.

FIGS. 5A, 5B, and 5C show that the major tyrosyl phosphorylated polypeptide of Flt4-transfected cells stimu-

lated with PC-3 conditioned medium is the 125 kD Flt4 polypeptide (VEGFR-3).

FIG. 6 shows Western analysis of the Flt4 ligand activity isolated from PC-3 conditioned medium.

FIG. 7 shows results of gel electrophoresis of chromatographic fractions from the affinity purification of Flt4 ligand (VEGF-C) isolated from PC-3 conditioned medium.

FIG. 8 shows results of Western analysis of Flt4 autophosphorylation induced by either the Flt4 ligand (VEGF-C), VEGF, or PlGF.

FIG. 9A schematically depicts the cloning and analysis of the Flt4 ligand, VEGF-C. The VEGF-C coding sequence (shaded boxes) and signal sequence (ss) are depicted between 5' and 3' untranslated (ut) nucleic acid regions.

FIGS. 9B-D show the nucleotide and deduced amino acid sequence of the coding portion of Flt4 ligand cDNA. The cleavage site for the putative signal peptide is indicated with a shaded triangle.

FIGS. 10A-D show a comparison of the deduced amino acid sequences of PDGF-A (SEQ ID NO: 36); PDGF-B (SEQ ID NO: 37); two PlGF isoforms (SEQ ID NOs: 38 and 39); four VEGF isoforms (SEQ ID NOs: 40-43); and Flt4 ligand (VEGF-C) (SEQ ID NO: 33).

FIG. 11 shows the stimulation of autophosphorylation of the Flt4 receptor by conditioned medium from cells transfected with the Flt4-L (VEGF-C) expression vector.

FIG. 12 shows Northern blotting analysis of Flt4-L (VEGF-C) mRNA in tumor cell lines and in brain tissue.

FIG. 13A is an autoradiograph showing recombinant VEGF-C isolated following a pulse-chase experiment and electrophoresed via SDS-PAGE under reducing conditions.

FIG. 13B is a photograph of polyacrylamide gel showing that recombinant VEGF-C forms are disulfide-linked in nonreducing conditions.

FIGS. 14A and 14B depict Western blots showing that VEGF-C stimulates autophosphorylation of VEGFR-2 (KDR) but has no effect on PDGFR- β phosphorylation.

FIGS. 15A and 15B show that VEGF-C stimulates endothelial cell migration in a three-dimensional collagen gel assay.

FIG. 16A shows the expression of VEGF-C mRNA in human adult tissues.

FIG. 16B shows the expression of VEGF, VEGF-B, and VEGF-C in selected human fetal tissues.

FIG. 17 schematically depicts the chromosomal localization of the VEGF-C gene.

FIG. 18 is a Northern blot hybridization study showing the effects of hypoxia on the mRNA expression of VEGF-A, VEGF-B and VEGF-C.

DETAILED DESCRIPTION OF THE INVENTION

Described herein is the isolation of a novel vascular endothelial growth factor and the cloning of a cDNA encoding this growth factor from a cDNA library prepared from the human prostatic adenocarcinoma cell line PC-3. The isolated cDNA encodes a protein which is proteolytically processed and secreted to cell culture medium. The secreted protein, designated VEGF-C, binds to the extracellular domain of Flt4 (designated VEGFR-3) and induces tyrosine autophosphorylation of Flt4 and VEGFR-2. VEGF-C also stimulates the migration of endothelial cells in collagen gel.

The present invention also is directed to novel growth factors which are ligands for the Flt4 receptor tyrosine

kinase (VEGFR-3). Ligands of the invention are members of a family of platelet-derived growth factors/vascular endothelial growth factors which promote mitosis and proliferation of vascular endothelial cells and/or mesodermal cells. Ligands recognizing the Flt4 receptor tyrosine kinase were purified from a PC-3 prostatic adenocarcinoma cell line (ATCC CRL1435). When applied to a population of cells expressing the Flt4 receptor, ligands of the invention stimulate autophosphorylation, resulting in receptor activation. The invention also provides inhibitors of the Flt4 receptor, including antibodies directed against the ligand. A ligand according to the invention may be coexpressed as a larger precursor which is cleaved to produce the ligand. A coexpressed region in some cases results from alternative splicing of RNA of the ligand gene. Such a co-expressed region may be a function of the particular expression system used to obtain the ligand. The skilled artisan understands that in recombinant production of proteins, additional sequence may be expressed along with a functional peptide depending upon the particular recombinant construct used to express the protein, and subsequently removed to obtain the desired ligand. In some cases the recombinant ligand can be made lacking certain residues of the endogenous/natural ligand. Moreover, it is well-known in that conservative replacements may be made in a protein which do not alter the function of the protein. Accordingly, it is anticipated that such alterations are within the scope of the claims. It is intended that the precursor sequence shown in SEQ ID NO: 33 is capable of stimulating the Flt4 ligand without any further processing in a manner similar to that in which VEGF stimulates its receptor in its unprocessed form.

Results reported herein show that VEGFR-3 transmits signals for a novel growth factor. This conclusion is based on the specific binding of VEGF-C to recombinant Flt4EC (Flt4 extracellular domain) protein and the induction of VEGFR-3 autophosphorylation by medium from VEGF-C transfected cells. In contrast, VEGF and PlGF did not show specific binding to VEGFR-3 or induce its autophosphorylation.

A major part of the difference in the observed molecular mass of the purified and recombinant VEGF-C and the deduced molecular mass of the VEGF-C encoded by the VEGF-C open reading frame (ORF) may be due to proteolytic removal of sequences in the carboxyl terminal region of the latter. Proteolytic processing of the VEGF-C precursor may occur at more than one cleavage site because the 32 kD molecular mass of the recombinant secreted ligand was also less than the deduced molecular mass of VEGF-C ORF without the signal peptide. By extrapolation from studies of the structure of PDGF (Heldin, et al., *Growth Factors*, 8:245-52 (1993)), one can speculate that the region critical for receptor binding and activation by VEGF-C is contained within the amino-terminal first 180 or so amino acid residues of the secreted VEGF-C protein lacking the signal sequence. In fact, the region critical for receptor binding and activation by VEGF-C is believed to be contained within the first approximately 120 amino acid residues of the secreted VEGF-C protein lacking the signal sequence. Thus, the 23 kD polypeptide binding VEGFR-3 is likely to represent the VEGF-homologous domain. After biosynthesis, the nascent VEGF-C polypeptide may be glycosylated at three putative N-linked glycosylation sites identified in the deduced VEGF-C amino acid sequence:

The carboxyl terminal amino acid sequences, which increase the length of the VEGF-C polypeptide in comparison with other ligands of this family, show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring 3 protein (BR3P) sequence (Dignam and Case, *Gene*,

88:133-40 (1990); Paulsson, et al., *J. Mol. Biol.*, 211:331-49 (1990)). This novel C-terminal silk protein-like structural motif of VEGF-C may fold into an independent domain, which, on the basis of the considerations above, is at least partially cleaved off after biosynthesis. Interestingly, at least one cysteine motif of the BR3P type is also found in the carboxyl terminus of VEGF. In our experiments both the putative precursor and cleaved ligand were detected in the cell culture media, although processing was apparently cell-associated on the basis of the pulse-chase experiments. The determination of the amino terminal sequence of the isolated carboxyl terminal fragment will allow the identification of the proteolytic processing site. The generation of antibodies against different parts of the VEGF-C molecule will allow the exact determination of the precursor-product relationship and ratio, their cellular distribution, and the kinetics of processing and secretion.

VEGF-C has a conserved pattern of eight cysteine residues, which may participate in the formation of intra- and interchain disulfide bonds, creating an antiparallel dimeric biologically active molecule, similar to PDGF. Mutational analysis of the cysteine residues involved in the interchain disulfide bridges has shown that, in contrast to PDGF, VEGF dimers need to be held together by these covalent interactions in order to maintain biological activity. Disulfide linking of the VEGF-C polypeptide chain was evident in the analysis of VEGF-C in nonreducing conditions.

VEGFR-3, which thus distinguishes between VEGF and VEGF-C, is closely related in structure to VEGFR-1 and VEGFR-2. Finnerty, et al., *Oncogene*, 8:2293-98 (1993); Galland, et al., *Oncogene*, 8:1233-40 (1993); Pajusola, et al., *Cancer Res.*, 52:5738-43 (1992). However, the mature form of VEGFR-3 differs from the two other VEGFRs in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola, et al., *Oncogene*, 9:3545-55 (1994). Another difference is that the 4.5 and 5.8 kb VEGFR-3 mRNAs encode polypeptides differing in their C-termini and apparently in their signalling properties due to the use of alternative 3' exons. Borg et al., *Oncogene*, 10:973-84 (1995); Pajusola et al., *Oncogene*, 8:2931-37 (1993).

Besides VEGFR-3, VEGFR-2 tyrosine kinase also is activated in response to VEGF-C. VEGFR-2 mediated signals cause striking changes in the morphology, actin reorganization and membrane ruffling of porcine aortic endothelial cells overexpressing this receptor. In these cells, VEGFR-2 also mediated ligand-induced chemotaxis and mitogenicity. Waltenberger et al., *J. Biol. Chem.*, 269:26988-95 (1994). Similarly, the receptor chimera CSF-1R/VEGFR-3 was mitogenic when ectopically expressed in NIH3T3 fibroblastic cells, but not in porcine aortic endothelial cells (Pajusola et al., 1994). Consistent with such results, the bovine capillary endothelial cells (BCE), which express VEGFR-2 mRNA but very little or no VEGFR-1 or VEGFR-3 mRNAs, showed enhanced migration when stimulated with VEGF-C. As shown here, light microscopy of the BCE cell cultures in collagen gel also suggested that VEGF-C stimulated the proliferation of these cells. The already existing data thus indicate that the VEGF ligands and receptors show a great specificity in their signalling, which may be cell type dependent.

The expression pattern of the VEGFR-3 (Kaipainen et al., *Proc. Natl. Acad. Sci. USA*, 92:3566-70 (1995)) suggests that VEGF-C may function in the formation of the venous and lymphatic vascular systems during embryogenesis. Constitutive expression of VEGF-C in adult tissues shown

herein further suggests that this gene product also is involved in the maintenance of the differentiated functions of the lymphatic endothelium where VEGFR-3 is expressed (Kaipainen et al., 1995). Lymphatic capillaries do not have well formed basal laminae and an interesting possibility remains that the silk-like BR3P motif is involved in producing a supramolecular structure which could regulate the availability of VEGF-C in tissues. However, as shown here, VEGF-C also activates VEGFR-2, which is abundant in proliferating endothelial cells of vascular sprouts and branching vessels of embryonic tissues, but decreased in adult tissues. Millauer et al., *Nature*, 367:576-78 (1993). These data have suggested that VEGFR-2 is a major regulator of vasculogenesis and angiogenesis. VEGF-C may thus have a unique effect in lymphatic endothelium and a more redundant function shared with VEGF in angiogenesis and possibly permeability regulation of several types of endothelia. Because VEGF-C stimulates VEGFR-2 and promotes endothelial migration, a utility for VEGF-C is suggested as an inducer of angiogenesis of blood and lymphatic vessels in wound healing, tissue transplantation, in eye diseases, in the formation of collateral vessels around arterial stenoses and into injured tissues after infarction.

Taken together, these results show an increased complexity of signalling in the vascular endothelium. They reinforce the concept that when organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases in several types of functionally and morphologically distinct vessels. However, upon suitable angiogenic stimuli, endothelial cells can re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their tissue environment. This process of angiogenesis concurrent with tissue development and regeneration depends on the tightly controlled balance between positive and negative signals for endothelial cell proliferation, migration, differentiation and survival. Previously-identified growth factors promoting angiogenesis include the fibroblast growth factors, hepatocyte growth factor/scatter factor, PDGF and TGF- α . (See, e.g., Folkman, *Nature Med.* 1:27-31 (1995); Friesel and Maciag, *FASEB J.* 9:919-25 (1995); Mustonen and Alitalo, *J. Cell Biol.* 129:895-98 (1995). However, VEGF has been the only growth factor relatively specific for endothelial cells. The newly identified factors VEGF-B and VEGF-C thus increase our understanding of the complexity of the specific and redundant positive signals for endothelial cells involved in vasculogenesis, angiogenesis, permeability and perhaps also other endothelial functions.

Also described herein is the localization of the VEGF-C gene in human chromosomes by analysis of somatic cell hybrids and fluorescence in situ hybridization (FISH). Southern blotting and polymerase chain reaction analysis of somatic cell hybrids and fluorescence in situ hybridization of metaphase chromosomes was used to assess the chromosomal localization of the VEGF-C gene. The VEGF-C gene was located on chromosome 4q34, close to the human aspartylglucosaminidase gene previously mapped to 4q34-35. The VEGF-C locus in 4q34 is a candidate target for mutations leading to vascular malformations or cardiovascular diseases. Expression studies by Northern blotting and hybridization show abundant VEGF-C expression in heart and skeletal muscle; other tissues, such as lung and kidney, also express this gene. Whereas PIGF is predominantly expressed in the placenta, the expression patterns of the three VEGFs overlap in many tissues, which suggests that they may form heterodimers and interact to exert their physiological functions.

Targeted mutagenesis leading to inactivation of the VEGF receptor loci in the mouse genome has shown that VEGFR-1 is necessary for the proper organization of endothelial cells forming the vascular endothelium, while VEGFR-2 is necessary for the generation of both endothelial and hematopoietic cells. This suggests that the four genes of the VEGF family can be targets for mutations leading to vascular malformations or cardiovascular diseases.

The following Examples illustrate preferred embodiments of the invention, wherein the isolation, characterization, and function of Flt4 ligands and ligand-encoding nucleic acids according to the invention are shown.

EXAMPLE 1

Production of pLTRFlt4 Expression Vector

Construction of the LTR-Flt4 vector is schematically shown in FIGS. 2A and 2B. The full-length Flt4s cDNA (Genbank Accession No. X68203) was assembled by first subcloning the S2.5 fragment, reported in Pajusola et al., *Cancer Res.* 52:5738-5743 (1992), incorporated by reference herein, containing base pairs 56-2534 of the Flt4s into the EcoRI site of the pSP73 vector (Promega, Madison, Wis.).

Since cDNA libraries used for screening of Flt4 cDNAs did not contain its most 5' protein-coding sequences, inverse PCR was used for the amplification of the 5' end of Flt4 corresponding to the first 12 amino acid residues (MQRGAAALCLRLW). Poly(A)⁺ RNA was isolated from HEL cells and double-stranded cDNA copy was synthesized using the Amersham cDNA Synthesis System Plus kit and a gene specific primer: 5'-TGTCTCGCTGTGCTTGTCT-3' (SEQ ID NO: 1), which was located 195 bp downstream of the 5' end of clone S2.5. Double stranded cDNA was treated with T4 DNA polymerase to blunt the ends and cDNA was purified with Centricon 100 filters (Amicon Inc., Beverly, Mass.). Circularization was made in a total volume of 150 μ l. The reaction mixture contained ligation buffer, 5% PEG-8000, 1 mM DTT and 8U of T4 DNA ligase (New England Biolabs). Ligation was carried out at 16° C. for 16 hours. Fifteen μ l of this reaction mix was used in a standard 100 μ l PCR reaction containing 100 ng of specific primers including SacI and PstI restriction sites, present in this segment of the Flt4 cDNA, and 1 unit of Taq DNA polymerase (Perkin Elmer Cetus). Two rounds of PCR were performed using 33 cycles (denaturation at 95° C. for 1 minute, annealing at 55° C. for 2 minutes and elongation at 72° C. for 4 minutes). The PCR mixture was treated sequentially with the SacI and PstI restriction enzymes and after purification with MagicPCR Preps (Promega) DNA fragments were subcloned into the pGEM3Z(+) vector for sequencing. The sequence obtained corresponds to the 5' end of the Flt4s cDNA clone deposited in the Genbank Database as Accession No. X68203.

The sequence encoding the first 12 amino acid residues was added to the expression construct by ligating an SphI digested PCR fragment amplified using reverse transcription-PCR of poly(A)⁺ RNA isolated from the HEL cells using the oligonucleotides 5'-ACATGCATGC CAC-CATGCAG CGGGGCGCCG CGCTGTGCTT GCGACTGTGG CTCTGCCTGG GACTCCTGGA-3' (SEQ ID NO: 2) forward primer, SphI site underlined, the translational start codon marked in bold follows an optimized Kozak consensus sequence Kozak, *Nucl. Acids Res.* 15: 8125-8148, 1987) and 5'-ACATGCATGC CCGCCGGT CATCC-3' (SEQ ID NO: 3) (reverse primer, SphI site underlined) to the 5' end of the S2.5 fragment, thus replacing

unique SphI fragment of the S2.5 plasmid. The resulting vector was digested with EcoRI and ClaI and ligated to a 138 bp PCR fragment amplified from the 0.6 kb EcoRI fragment (base pairs 3789 to 4416 in the Genbank X68203 sequence) which encodes the 3' end of Flt4s shown in FIG. 1 of Pajusola et al., *Cancer Res.* 52:5738-5743, 1992, using the oligonucleotides 5'-CGGAATTCCTCC CATGACCCCAAC-3' (SEQ ID NO: 4) (forward, EcoRI site underlined) and 5'-CCATCGATGG ATCTACCTG AAGCCGCTTT CTT-3' (SEQ ID NO: 5) (reverse, ClaI site underlined). The coding domain was completed by ligation of the 1.2 kb EcoRI fragment (base pairs 2535-3789 of sequence X68203) into the above construct. The complete cDNA was subcloned as a HindIII-ClaI (blunted) fragment (this ClaI site was also included in the 3' primer used to construct the 3' end of the coding sequence) to the pLTRpoly expression vector reported in Mäkelä et al., *Gene*, 118: 293-294 (1992) (Genbank accession number X60280), incorporated by reference herein, using its HindIII-Acc I (blunted), restriction sites.

The long form of Flt4 was produced by replacing the 3'-end of the short form as follows: The 3' region of the Flt4 cDNA was PCR-amplified using a gene specific and a pGEM 3Z vector specific (SP6 promoter) oligonucleotide 5'-ATTAGGTGACACTATA-3' (SEQ ID NO: 6) as reverse and forward primers, respectively, and an Flt4 cDNA clone containing a 495 bp EcoRI fragment extending downstream of the EcoRI site at nucleotide 3789 of the Genbank X68203 sequence (the sequence downstream of this EcoRI site is deposited as the Flt4 long form 3' sequence having Genbank accession number S66407). The gene specific oligonucleotide contained a BamHI restriction site located right after the end of the coding region. The sequence of that (reverse primer) oligonucleotide was 5'-CCATCGATGGATCCCGATGCTGCTTAGTAGCTG-3' (SEQ ID NO: 7) (BamHI site is underlined). The PCR product was digested with EcoRI and BamHI and transferred in frame to LTRFlt4s vector fragment from which the coding sequences downstream of the EcoRI site at base pair 2535 (see sequence X68203) had been removed by EcoRI-BamHI digestion. Again, the coding domain was completed by ligation of the 1.2 kb EcoRI fragment (base pairs 2535-3789 of sequence X68203) back into the resulting construct.

EXAMPLE 2

Production and Analysis of Flt4 Transfected Cells

NIH3T3 cells (60% confluent) were co-transfected with 5 µg of the pLTRFlt4 construct and 0.25 µg of the pSV2neo vector (ATCC) containing the neomycin phosphotransferase gene, using the DOTAP liposome-based transfection reagents (Boehringer Mannheim, Mannheim, Germany). One day after the transfection the cells were transferred into selection media containing 0.5 mg/ml geneticin (GIBCO, Grand Island, N.Y.). Colonies of geneticin-resistant cells were isolated and analyzed for expression of the Flt4 proteins. Cells were lysed in boiling lysis buffer containing 3.3% SDS (sodium dodecyl sulphate), 125 mM Tris, pH 6.8. Protein concentrations of the samples were measured by the BCA method (Pierce, Rockford, Ill.). About 50 µg of protein of each lysate were analyzed for the presence of Flt4 by 6% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using antisera against the carboxyl terminus of Flt4 and the ECL method (Amersham).

For production of anti-Flt4 antiserum the Flt4 cDNA fragment encoding the 40 carboxy-terminal amino acid residues of the short form: NH2- PMTPTTYKG SVDN-

QTDSGM VLASEEFEQ! ESRHRQESGFR-COOH (SEQ ID NO: 8) was cloned as a 657 bp EcoRI-fragment into the pGEX-1AT bacterial expression vector (Pharmacia) in frame with the glutathione-S-transferase coding region. The resultant GST-Flt4s fusion protein was produced in *E. coli* and purified by affinity chromatography using a glutathione-Sepharose 4B column. The purified protein was lyophilized, dissolved in phosphate buffered saline (PBS), mixed with Freund's adjuvant and used for immunization of rabbits at biweekly intervals using methods standard in the art (Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1988). Antisera were used after the fourth booster immunization for immunoprecipitation of Flt4 from the transfected cells and cell clones expressing Flt4 were used for ligand stimulation analysis.

EXAMPLE 3

Construction of a Flt4 EC Baculovirus Vector and Expression and Purification of its Product

The construction of an Flt4 extracellular domain (EC) baculovirus vector is schematically shown in FIG. 3. The Flt4-encoding cDNA has been prepared in both a long form and a short form, each being incorporated in a vector under control of the Moloney murine leukemia virus LTR promoter. The nucleotide sequence of the short form of the Flt4 receptor is available on the Genbank database as Accession No. X68203 and the specific 3' segment of the long form cDNA is available as Accession No. S66407.

The ends of a cDNA segment encoding Flt4 extracellular domain (EC) were modified as follows: The 3' end of Flt4 cDNA sequence (Genbank Accession Number X68203) which encodes the extracellular domain was amplified using primer 1116 5'-CTGGAGTCGACTTGGCCGACT-3' (SEQ ID NO: 9, SalI site underlined) and primer 1315 5'-CGCGGATCCCTAGTGATGGTGATGCTGTCTACCTTCGATCATGCT GCCCTTAT CCTC-3' (SEQ ID NO: 10, BamHI site underlined). The sequence complementary to that of primer 1315 continues after the Flt4 reading frame and encodes 6 histidine residues for binding to a Ni-NTA column (Qiagen, Hilden, Germany) followed by a stop codon, and an added Bam HI site. The amplified fragment was digested with SalI and BamHI and used to replace a unique SalI-BamHI fragment in the LTRFlt4 vector shown in FIG. 3. The SalI-BamHI fragment that was replaced encodes the Flt4 transmembrane and cytoplasmic domains.

The 5' end without the Flt4 signal sequence encoding region was amplified by PCR using the primer. 1335 5'-CCCAAGCTTGGATCCAAAGTGCGTACTCCATGACC-3' (SEQ ID NO: 11) (the primer contains added HindIII (AAGCTT) and BamHI (GGATCC) restriction sites, which are underlined) and primer 1332 5'-GTTGCCTGTGATGTGCACCA-3' (SEQ ID NO: 12). The amplified fragment was digested with HindIII and SphI (the HindIII site (AAGCTT) is underlined in primer 1335 and the SphI site is within the amplified region of the Flt4 cDNA). The resultant HindIII-SphI fragment was used to replace a HindIII-SphI fragment in the modified LTRFlt4 vector described immediately above (the HindIII site is in the 5' junction of the Flt4 insert with the pLTRpoly portion of the vector, the SphI site is in Flt4 cDNA). The resultant Flt4EC insert was then ligated as a BamHI fragment into the BamHI site in the pVTBac plasmid as disclosed in Tessier et al., *Gene* 98: 177-183 (1991), incorporated by reference herein. The orientation was confirmed to be correct by partial sequencing so that the open reading frame of the signal sequence-encoding portion of the vector continued in

frame with the Flt4 sequence. That construct was transfected together with the baculovirus genomic DNA into SF-9 cells by lipofection. Recombinant virus was purified, amplified and used for infection of High-Five cells (Invitrogen, San Diego, Calif.) using methods standard in the art. The Flt4 extracellular domain (Flt4EC) was purified from the culture medium of the infected High-Five cells using Ni-NTA affinity chromatography according to manufacturer's instructions (Qiagen) for binding and elution of the 6xHis tag encoded in the COOH-terminus of the recombinant Flt4 extracellular domain.

EXAMPLE 4

Isolation of Flt4 Ligand from Conditioned Media

An Flt4 ligand according to the invention was isolated from conditioned media from PC-3 prostatic adenocarcinoma cell line CRL1435 from the American Type Culture Collection and cultured as instructed by the supplier in Ham's F-12 Nutrient mixture (GIBCO) containing 7% fetal calf serum. In order to prepare the conditioned media, confluent PC-3 cells were cultured for 7 days in Ham's F-12 Nutrient mixture (GIBCO) in the absence of fetal bovine serum. Medium was then cleared by centrifugation at 10,000 g for 20 minutes. The medium was then screened to determine its ability to induce tyrosine phosphorylation of Flt4 by exposure to NIH3T3 cells which had been transfected with Flt4-encoding cDNA using the pLTRFlt41 vector. For receptor stimulation experiments, subconfluent NIH3T3 cells were starved overnight in serum-free DMEM medium (GIBCO) containing 0.2% BSA. The cells were stimulated with the conditioned media for 5 minutes, washed twice with cold PBS containing 100 μ M vanadate and lysed in RIPA buffer (10 mM Tris pH 7.5, 50 mM NaCl, 0.5% sodium deoxycholate, 0.5% Nonidet P40 (BDH, Poole, England), 0.1% SDS, 0.1 U/ml Aprotinin (Boehringer Mannheim), 1 mM vanadate) for receptor immunoprecipitation analysis. The lysates were centrifuged for 20 minutes at 15,000 \times g. The supernatants were incubated for 2 hours on ice with 3 μ l of the antiserum against the Flt4 C-terminus described in Example 2 and also in Pajusola, et al. *Oncogene* 8: 2931-2937, (1993), incorporated by reference herein.

After a 2 hour incubation in the presence of anti-Flt4 antiserum, protein A-Sepharose (Pharmacia) was added and incubation was continued for 45 minutes with rotation. The immunoprecipitates were washed three times with the immunoprecipitation buffer and twice with 10 mM Tris, pH 7.5 before analysis in SDS-PAGE. Polypeptides were transferred to nitrocellulose and analyzed by Western blotting using Flt4- or phosphotyrosine-specific antisera and the ECL method (Amersham International, Buckinghamshire, England). Anti-phosphotyrosine monoclonal antibodies (anti-PTyr: PY20) were purchased from Transduction Laboratories (Lexington, Ky.). In some cases, the filters were restained with a second antibody after stripping. The stripping of the filters was done for 30 minutes at 5 $^{\circ}$ C. in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7 with occasional agitation.

As shown in FIG. 4, the PC-3 cell conditioned medium stimulated tyrosine phosphorylation of a 125 kD polypeptide when Flt4-expressing NIH3T3 cells were treated with the indicated preparations of media, lysed, and the lysates were immunoprecipitated with anti-Flt4 antiserum followed by SDS-PAGE, Western blotting, and staining using anti-PTyr antibodies. The resulting band was weakly phosphorylated upon stimulation with unconcentrated PC-3 conditioned medium (lane 2). The 125 kD band comigrated with

the tyrosine phosphorylated, processed form of the mature Flt4 from pervanadate-treated cells (compare lanes 2 and 7 of FIG. 4, see also FIG. 5A). Comigration was confirmed upon restaining with anti-Flt4 antibodies as is also shown in FIG. 5A (panel on the right). In order to show that the 125 kD polypeptide is not a non-specific component of the conditioned medium reactive with anti-phosphotyrosine antibodies, 15 μ l of conditioned medium were separated by SDS-PAGE, blotted on nitrocellulose and the blot was stained with anti-PTyr antibodies. No signal was obtained (FIG. 5B). Also, unconditioned medium failed to stimulate Flt4 phosphorylation, as shown in FIG. 4, lane 1.

As shown in FIG. 4, lane 3, stimulating activity was considerably increased when the PC-3 conditioned medium was concentrated four-fold using a Centricon-10 concentrator (Amicon). FIG. 4, lane 4, shows that pretreatment of the concentrated PC-3 conditioned medium with 50 μ l of the Flt4 extracellular domain coupled to CNBr-activated sepharose CL-4B (Pharmacia; about 1 mg of Flt4EC domain/ml sepharose resin) completely abolished Flt4 tyrosine phosphorylation. Similar pretreatment of the conditioned medium with unsubstituted sepharose CL-4B did not affect stimulatory activity, as shown in FIG. 4, lane 5. Also, the flow through obtained after concentration, which contained proteins of less than 10,000 molecular weight, did not stimulate Flt4 phosphorylation, as shown in FIG. 4, lane 6.

The foregoing data show that PC-3 cells produce a ligand which binds to the extracellular domain of Flt4 and activates this receptor.

EXAMPLE 5

Purification of the Flt4 Ligand

The ligand expressed by PC-3 cells as characterized in Example 4 was purified and isolated using a recombinantly-produced Flt4 extracellular domain (Flt4EC) in affinity chromatography.

Two harvests of serum-free conditioned medium, comprising a total of 8 L, were collected from 500 confluent 15 cm diameter culture dishes containing confluent layers of PC-3 cells. The conditioned medium was clarified by centrifugation at 10,000 \times g and concentrated 80-fold using an Ultrasette Tangential Flow Device (Filtron, Northborough, Mass.) with a 10 kD cutoff Omega Ultrafiltration membrane according to the manufacturer's instructions. Recombinant Flt4 extracellular domain was expressed in a recombinant baculovirus cell system and purified by affinity chromatography on Ni-agarose (Ni-NTA affinity column obtained from Qiagen). The purified extracellular domain was coupled to CNBr-activated Sepharose CL-4B at a concentration of 5 mg/ml and used as an affinity matrix for ligand affinity chromatography.

Concentrated conditioned medium was incubated with 2 ml of the recombinant Flt4 extracellular domain-Sepharose affinity matrix in a rolling tube at room temperature for 5 hours. All subsequent purification steps were at 4 $^{\circ}$ C. The affinity matrix was then transferred to a column (Pharmacia) with an inner diameter of 15 mm and washed successively with 100 ml of PBS and 50 ml of 10 mM Na-phosphate buffer (pH 6.8). Bound material was eluted step-wise with 100 mM glycine-HCl, successive 6 ml elutions having pHs of 4.0, 2.4, and 1.9. Several 2 ml fractions of the eluate were collected in tubes containing 0.5 ml 1 M Na-phosphate (pH 8.0). Fractions were mixed immediately and dialyzed in 1 mM Tris-HCl (pH 7.5). Aliquots of 75 μ l each were analyzed

for their ability to stimulate tyrosine phosphorylation of Flt4. The ultrafiltrate, 100 μ l aliquots of the concentrated conditioned medium before and after ligand affinity chromatography, as well as 15-fold concentrated fractions of material released from the Flt4 extracellular domain-Sepharose matrix during the washings were also analyzed for their ability to stimulate Flt4 tyrosine phosphorylation.

As shown in FIG. 6, lane 3, the concentrated conditioned medium induced prominent tyrosine phosphorylation of Flt4 in transfected NIH3T3 cells overexpressing Flt4. This activity was not observed in conditioned medium taken after medium was exposed to the Flt4 Sepharose affinity matrix described above (lane 4). The specifically-bound Flt4-stimulating material was retained on the affinity matrix upon washes in PBS, 10 mM Na-phosphate buffer (pH 6.8), and at pH 4.0 (lanes 5-7, respectively), and it was eluted in the first two 2 ml aliquots at pH 2.4 (lanes 8 and 9). A further decrease of the pH of the elution buffer did not cause release of additional Flt4-stimulating material (lane 11).

Small aliquots of the chromatographic fractions were concentrated in a SpeedVac concentrator (Savant, Farmingdale, N.Y.) and subjected to SDS-PAGE under reducing conditions with subsequent silver staining of the gel. As shown in FIG. 7, the major polypeptide, having a molecular weight of approximately 23 kD (reducing conditions), was detected in the fractions containing Flt4 stimulating activity (corresponding to lanes 8 and 9 in FIG. 6). That polypeptide was not found in the other chromatographic fractions. On the other hand, all other components detected in the two active fractions were also distributed in the starting material and in small amounts in the other washing and elution steps after their concentration. Similar results were obtained in three independent affinity purifications, indicating that the 23 kD polypeptide specifically binds to Flt4 and induces its tyrosine phosphorylation.

Fractions containing the 23 kD polypeptide were combined, dried in a SpeedVac concentrator and subjected to SDS-PAGE in a 12.5% gel. The proteins from the gel were then electroblotted to Immobilon-P (PVDF) transfer membrane (Millipore, Marborough, Mass.) and visualized by staining of the blot with Coomassie blue R-250. The region containing only the stained 23 kD band was cut from the blot and was subjected to N-terminal amino acid sequence analysis in a Prosite Protein Sequencing System (Applied Biosystems, Foster City, Calif.). The data were analyzed using a 610A Data Analysis System (Applied Biosystems). Analysis revealed a single N-terminal sequence of NH₂-XEETIKFAAAHYNTEILK-COOH (SEQ ID NO: 13).

EXAMPLE 6

Construction of PC-3 Cell cDNA Library in a Eukaryotic Expression Vector

Poly(A)⁺ RNA was isolated from five 15 cm-diameter confluent dishes of PC-3 cells by a single step method using oligo(dT) (Type III, Collaborative Research) cellulose affinity chromatography (Sambrook et al., Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989). The yield was 70 μ g. Six micrograms of the Poly(A)⁺ RNA were used to prepare an oligo(dT)-primed cDNA library in the mammalian expression vector pcDNA I and the Librarian kit of Invitrogen according to the instructions included in the kit. The library was estimated to contain about 10⁶ independent recombinants with an average insert size of approximately 1.8 kb.

EXAMPLE 7

Amplification of the Unique Nucleotide Sequence Encoding the Flt4 Ligand

Degenerate oligonucleotides were designed based on the N-terminal amino acid sequence of the isolated Flt4 ligand and were used as primers in a polymerase chain reaction (PCR) to amplify cDNA encoding the Flt4 ligand from a PC-3 cell library. The overall strategy is schematically depicted in FIG. 9A, where the different primers have been marked with arrows.

The PCR was carried out using 1 μ g of DNA from the amplified PC-3 cDNA library and a mixture of sense-strand primers comprising 5'-GCAGARGARACNATHAA-3' (SEQ ID NO: 14) (wherein R is A or G, N is A, G, C or T and H is A, C or T), encoding amino acid residues 2-6 (EETIK, SEQ ID NO: 15) and antisense-strand primers 5'-GCAYTTNARDATYTCNGT-3' (SEQ ID NO: 16) (wherein Y is C or T and D is A, G or T), corresponding to amino acid residues 14-18 (TEILK, SEQ ID NO: 17). Three extra nucleotides (GCA) were added to the 5'-terminus of each primer to increase annealing stability. Two successive PCR runs were carried out using 1 U per reaction of DynaZyme (F-500L, Finnzymes), a thermostable DNA polymerase, in a buffer supplied by the manufacturer (10 mM Tris-HCl, pH 8.8 at 25° C., 1.5 mM MgCl₂, 50 mM KCl, 0.1% Triton-X100), at an extension temperature of 72° C. The first PCR run was carried out for 43 cycles. The first three cycles were run at an annealing temperature of 33° C. for 2 minutes, and the remaining cycles were run at 42° C. for 1 minute.

The region of the gel containing a weak band of the expected size (57 bp) was cut out from the gel and eluted. The eluted material was reamplified for 30 cycles using the same primer pairs described above at 42° C. for 1 minute. The amplified fragment was cloned into a PCR II vector (Invitrogen) using the TA cloning kit (Invitrogen) and sequenced using the radioactive dideoxynucleotide sequencing method of Sanger. Six clones were analyzed and all contained the sequence encoding the expected peptide (amino acids 2-18 of the Flt4 ligand precursor). Nucleotide sequence spanning the region from the third nucleotide of codon 6 to the third nucleotide of codon 13 (the extension region) was identical in all six clones: 5'-ATTCGCTGCAGCACACTACAAC-3' (SEQ ID NO: 18) and thus was considered to represent an amplified product from the unique sequence encoding part of the amino terminus of the Flt4 ligand.

EXAMPLE 8

Amplification of the 5'-end of the cDNA Encoding the Flt4 Ligand

Based on the unique nucleotide sequence encoding the N-terminus of the isolated Flt4 ligand, two pairs of nested primers were designed to amplify, in two subsequent PCR-reactions, the complete 5'-end of the corresponding cDNAs from one microgram of DNA from the above-described PC-3 cDNA library. First, amplification was performed with primer 5'-TCNGTGTGTAGTGTGCTG-3' (SEQ ID NO: 19), which is the antisense-strand primer corresponding to amino acid residues 9-15 (AAHYNTE, SEQ ID NO: 20), and sense-strand primer 5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO: 21), corresponding to the T7 RNA promoter of the pcDNA1 vector used for construction of the library. "Touchdown"

17

PCR was used as disclosed in Don, et al., *Nucl. Acids Res.*, 19: 4008 (1991), incorporated by reference herein. The annealing temperature of the two first cycles was 62° C. and subsequently the annealing temperature was decreased in every other cycle by 1° C. until a final temperature of 53° C. was reached, at which temperature 16 additional cycles were conducted. Annealing time was 1 minute and extension at each cycle was conducted at 72° C. for 1 minute. Multiple amplified DNA fragments were obtained in the first reaction. The products of the first amplification (1 ul of a 1:100 dilution in water) were used in the second amplification reaction employing the nested primers 5'-GTTGTAGTGTGCTGCAGCGAATT-3' (SEQ ID NO: 22), an antisense-strand primer corresponding to amino acid residues 6-13 (KFAAAHYN, SEQ ID NO: 23) of the Flt4 ligand, and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 24), a sense-strand primer corresponding to nucleotides 2179-2199 of the pcDNA1 vector. The sequences of these sense and antisense primers overlapped with the 3' ends of the corresponding primers used in the first PCR. "Touchdown" PCR was carried out by decreasing the annealing temperature from 72° C. to 66° C. and continuing with 18 additional cycles at 66° C. The annealing time was 1 minute and extension at each cycle was carried out at 72° C. for 2 minutes. One major product of about 220 bp and three minor products of about 270 bp, 150 bp, and 100 bp were obtained.

The amplified fragment of approximately 220 bp was cut out from the agarose gel, cloned into a pCRII vector using the TA cloning kit (Invitrogen) and sequenced. Three recombinant clones were analyzed and they contained the sequence

5'-TCACTATAGGGAGACCCAAGCTTGGTACCGAGCTCGGATCCACTAGT AACGGCCGCCAGTGTGGTGAATTCGACGAACCTCATGACTGTACTCTACCCAGAATATTGGAAAATGTACAAGTGTCAAGTCAAGTCAAGGAGGCG

TGGCAACATAACAGAGAACAGGCCAACCTCAACTC AAGGACAGAAG AGACTATAAAATTCGCTGCAGCACACTACAAC-3' (SEQ ID NO: 25). The beginning of the sequence represents the pcDNA1 vector and the underlined sequence represents the amplified product of the 5'-end of the insert. The ATG codon located upstream of that sequence in the same reading frame is followed by an open reading frame containing the amplified product of the putative signal sequence and the first 13 amino acid residues of the secreted Flt4 ligand. The cloning of the 5' end of the Flt4 cDNA, as described in the preceding two examples, is depicted schematically in FIG. 9A.

EXAMPLE 9

Amplification of the 3'-end of cDNA Encoding the Flt4 Ligand

Based upon the amplified 5'-sequence of the clones encoding the Flt4 ligand, two pairs of non-overlapping nested primers were designed to amplify the 3'-portion of the FLT4-L clones. The sense-strand primers 5'-ACAGAGAACAGGCCAAC-3' (SEQ ID NO: 26) and antisense-strand primer 5'-TCTAGCATTTAGGTGACAC-3' (SEQ ID NO: 27) corresponding to nucleotides 2311-2329 of the pcDNA1 vector were used in a first "touchdown" PCR. The annealing temperature of the reaction was decreased 1° C. every two cycles from 72° C. to 52° C., at which temperature 15 additional cycles were carried out. The annealing time was 1 minute and extension at each cycle was carried out at 72° C. for 3 minutes. DNA fragments of several sizes were obtained in the first amplifica-

18

tion. Those products were diluted 1:200 in water and reamplified in PCR using the second pair of primers: 5'-AAGAGACTATAAAATTCGCTGCAGC-3' (SEQ ID NO: 28) and 5'-CCCTCTAGATGCATGCTCGA-3' (SEQ ID NO: 29) (antisense-strand primer corresponding to nucleotides 2279-2298 of the pcDNA1 vector). Two DNA fragments were obtained, having sizes of 1350 bp and 570 bp. Those fragments were cloned into a pCRII vector and the inserts of the clones were sequenced. Both of these fragments were found to contain sequences encoding an amino acid sequence homologous to the VEGF sequence.

EXAMPLE 10

Screening the PC-3 Cell cDNA Library Using the 5' PCR Fragment of Flt4 Ligand cDNA

A 219 bp 5'-terminal fragment of Flt4 ligand cDNA was amplified by PCR using the 5' PCR fragment described above and primers 5'-GTTGTAGTGTGCTGCAGCGAATTT-3' (antisense-strand primer, SEQ ID NO: 30) and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 31) (sense-primer corresponding to nucleotides 2179-2199 of the pcDNA1 vector). The amplified product was subjected to digestion with EcoRI (Boehringer Mannheim) to remove the portion of the DNA sequence amplified from the pcDNA1 vector and the resulting 153 bp fragment encoding the 5' end of the Flt4 ligand was labeled with [³²P]-dCTP using the Klenow fragment of *E. coli* DNA polymerase I (Boehringer Mannheim). That fragment was used as a probe for hybridization screening of the amplified PC-3 cell cDNA library.

Filter replicas of the library were hybridized with the radioactively labeled probe at 42° C. for 20 hours in a solution containing 50% formamide, 5xSSPE, 5xDenhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA. Filters were washed twice in 1xSSC, 0.1% SDS for 30 minutes at room temperature, then twice for 30 minutes at 65° C. and exposed overnight.

On the basis of autoradiography, 10 positive recombinant bacterial colonies hybridizing with the probe were chosen from the library. Plasmid DNA was purified from these colonies and analyzed by EcoRI and NotI digestion and agarose gel electrophoresis followed by ethidium bromide staining. The ten plasmid clones were divided into three groups on the basis of the presence of insert sizes of approximately 1.7, 1.9 and 2.1 kb, respectively. Inserts of plasmids from each group were sequenced using the T7 oligonucleotide as a primer and walking primers for subsequent sequencing reactions.

Sequence analysis showed that all clones contain the open reading frame encoding the NH₂-terminal sequence of the Flt4 ligand. Furthermore, the 2.1 and 1.9 kb clones also contained sequences encoding the signal sequence (FIG. 9A, SS). The 5' end of the 1.7 kb clone began within the signal sequence-encoding portion. Dideoxy sequencing was continued using walking primers in the downstream direction. An 1140 nucleotide portion of the sequence of the longest clone is shown in FIGS. 9B through 9D. As can be seen in that figure, after the putative signal sequence the open reading frame terminates in a TAA stop codon 318 amino acid residues further downstream from the 33 amino acid signal sequence. When compared with sequences in the GenBank Database, the predicted protein product of this reading frame was found to be homologous with the predicted amino acid sequences of the PDGF/VEGF family of growth factors, as shown in FIGS. 10A through 10D.

be identified by a number of methods standard in the art, such as heteroduplex analysis of cDNA and genomic DNA, which are subsequently characterized.

EXAMPLE 12

Expression of the Flt4L Gene

Expression of transcripts corresponding to the Flt4 ligand (VEGF-C) was analyzed by hybridization of Northern blots containing isolated poly(A)⁺ RNA from HT-1080 and PC-3 human tumor cell lines. The probe was the radioactively labelled insert of the 2.1 kb cDNA clone (specific activity 10⁸–10⁹ cpm/mg of DNA). The blot was hybridized overnight at 42° C. using 50% formamide, 5×SSPE buffer, 2% SDS, 10×Denhardt's solution, 100 mg/ml salmon sperm DNA and 1×10⁶ cpm of the labelled probe/ml. The blot was washed at room temperature for 2×30 minutes in 2×SSC containing 0.05% SDS, and then for 2×20 min at 52° C. in 0.1×SSC containing 0.1% SDS. The blot was then exposed at -70° C. for three days using intensifying screens and Kodak XAR film. Both cell lines expressed an Flt4 ligand mRNA of about 2.3 kb, as well as VEGF and VEGF-B mRNAs (FIG. 12).

EXAMPLE 13

VEGF-C Chains are Proteolytically Processed after Biosynthesis and Disulfide Linked

The predicted molecular mass of the secreted polypeptide, as deduced from the VEGF-C ORF specified in SEQ ID NOs: 32 and 33, is 35,881 kD, suggesting that VEGF-C mRNA may be first translated into a precursor, from which the mature ligand of 23 kD is derived by proteolytic cleavage.

To study this, metabolic labelling of 293 EBNA cells transfected with the VEGF-C construct was carried out by addition of 100 µCi/ml of Pro-mixTM L-[³⁵S] in vitro cell labelling mix (Amersham) to the culture medium devoid of cysteine and methionine. After two hours, the cell layers were washed twice with PBS and the medium was then replaced with DMEM-0.2% BSA. After 1, 3, 6, 12 and 24 hours of subsequent incubation, the culture medium was collected, clarified by centrifugation, and concentrated, and VEGF-C was bound to 30 µl of a slurry of Flt4EC-Sepharose overnight at +4° C., followed by three washes in PBS, two washes in 20 mM Tris-HCl (pH 7.5), alkylation, SDS-PAGE and autoradiography.

These experiments demonstrated that a putative precursor polypeptide of 32 kD apparent molecular mass was bound to the Flt4EC affinity matrix from the CM of metabolically labelled cells transfected with a VEGF-C expression vector (FIG. 13A). Increased amounts of a 23 kD receptor-binding polypeptide accumulated in the culture medium during a subsequent chase period of 3 h, but not thereafter (lanes 2–4 and data not shown), suggesting that the 23 kD form is produced by proteolytic processing, which is cell-associated and incomplete, at least in the transiently transfected cells. The arrows in FIG. 13A indicate the 32 kDa and 23 kDa polypeptides of secreted VEGF-C.

In a related experiment, VEGF-C isolated using Flt4EC-Sepharose after a 4 h continuous metabolic labelling was analyzed by polyacrylamide gel electrophoresis in non-reducing conditions (FIG. 13B). Higher molecular mass forms were observed under nonreducing conditions, suggesting that the VEGF-C polypeptides can form disulfide-linked dimers and/or multimers (arrows in FIG. 13B).

EXAMPLE 14

Stimulation of VEGFR-2 Autophosphorylation by VEGF-C

Conditioned medium (CM) from 293 EBNA cells transfected with the VEGF-C vector also was used to stimulate porcine aortic endothelial (PAE) cells expressing VEGFR-2. Pajusola et al., *Oncogene*, 9:3545–55 (1994); Waltenberger et al., *J. Biol. Chem.*, 269:26988–95 (1994). The cells were lysed and immunoprecipitated using VEGFR-2-specific antiserum (Waltenberger et al., 1994).

PAE-KDR cells (Waltenberger et al., 1994) were grown in Ham's F12 medium-10% fetal calf serum (FCS). Confluent NIH3T3-Flt4 cells or PAE-KDR cells were starved overnight in DMEM or Ham's F12 media, respectively, supplemented with 0.2% bovine serum albumin (BSA) and then incubated for 5 min. with the analyzed media. Recombinant human VEGF (R&D Systems) and PDGF-BB were used as a control stimulating agents. The cells were washed twice with ice-cold tris-buffered saline (TBS) containing 100 mM sodium orthovanadate and lysed in RIPA buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 U/ml aprotinin and 1 mM sodium orthovanadate. The lysates were sonicated, clarified by centrifugation at 16,000 g for 20 min. and incubated for 3–6 h on ice with 3–5 µl of antisera specific for Flt4 (Pajusola et al., 1993), VEGFR-2 or PDGFR-β (Claesson-Welsh et al., *J. Biol. Chem.*, 264:1742–47 (1989); Waltenberger et al., 1994). Immunoprecipitates were bound to protein A-Sepharose, washed three times with RIPA containing 1 mM PMSF, 1 mM sodium orthovanadate, twice with 10 mM Tris-HCl (pH 7.4) and subjected to SDS-PAGE in a 7% gel. Polypeptides were transferred to nitrocellulose by Western blotting and analyzed using PY20 phosphotyrosine-specific monoclonal antibodies (Transduction Laboratories) or receptor-specific antiserum and ECL method (Amersham).

The results of the experiment are presented in FIGS. 14A and 14B. As shown in FIG. 14A, PAE cells expressing VEGFR-2 were stimulated with 10- or 2-fold concentrated medium from mock-transfected 293-EBNA cells (lanes 1 and 2), or with 2-, 5- or 10-fold concentrated medium from 293-EBNA cell cultures expressing the recombinant VEGF-C (lanes 3–6). VEGFR-2 was immunoprecipitated with specific antibodies and analyzed by SDS-PAGE and Western blotting using phosphotyrosine antibodies. For comparison, the stimulations were carried out with non-conditioned medium containing 50 ng/ml of purified recombinant VEGF (lanes 7 and 8). Lanes 6 and 7 show stimulation with VEGF-C- or VEGF- containing media pretreated with Flt4EC. As depicted in FIG. 14B, PDGFR-β-expressing NIH3T3 cells were stimulated with non-conditioned medium (lane 1), 5-fold concentrated CM from mock-transfected (lane 2) or VEGF-C-transfected (lanes 3 and 4) cells, or with non-conditioned medium containing 50 ng/ml of recombinant human PDGF-BB (lane 5). Medium containing VEGF-C was also pretreated with recombinant Flt4EC (lane 4). PDGFR-β was immunoprecipitated with specific antibodies and analyzed by SDS-PAGE and Western blotting using phosphotyrosine antibodies with subsequent stripping and reprobing of the membrane with antibodies specific for PDGFR-β.

A basal level of tyrosine phosphorylation of VEGFR-2 was detected in cells stimulated by CM from the mock-transfected cells. A further concentration of this medium resulted in only a slight enhancement of VEGFR-2 phosphorylation (FIG. 14A lanes 1 and 2). CM containing

EXAMPLE 11

Stimulation of Flt4 Autophosphorylation by the Protein Product of the Flt4 Ligand Vector

The 2.1 kb insert of the Flt4-L clone in pcDNA1 vector containing the open reading frame encoding the sequence shown in FIGS. 9B through 9D (SEQ ID NO: 32) was cut out from the vector using HindIII and NotI restriction enzymes, isolated from a preparative agarose gel and ligated to the corresponding sites in the pREP7 expression vector (Invitrogen). The pREP7 vector containing the above cloned insert was transfected into 293-EBNA cells (Invitrogen) using the calcium phosphate transfection method (Sambrook et al., Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989). About 48 hours after transfection the medium of the transfected cells was changed to DMEM medium lacking fetal calf serum and incubated for 36 h. The thus conditioned medium was then collected, centrifuged at 5000xg for 20 minutes, the supernatant was concentrated 5-fold using Centrprep 10 (Amicon) and used to stimulate NIH3T3 cells expressing LTRFlt4L, as in Example 4. The cells were lysed, immunoprecipitated using anti-Flt4 antiserum and analyzed by Western blotting using anti-phosphotyrosine antibodies.

As can be seen from FIG. 11, lanes 1 and 3, the conditioned medium from two different dishes of the transfected cells stimulated Flt4 autophosphorylation in comparison with the medium from mock-transfected cells, which gave only background levels of phosphorylation of the Flt4 receptor (lane 2). When the concentrated conditioned medium was pre-absorbed with 20 µl of a slurry of Flt4EC domain coupled to Sepharose (see example 4), no phosphorylation was obtained (lane 4), showing that the activity responsible for Flt4 autophosphorylation was indeed the Flt4 ligand. Thus, these results demonstrate that the Flt4-L plasmid vector clone having an approximately 2.1 kb insert and containing the open reading frame shown in FIG. 9B is expressed into a Flt4 ligand in cells transfected with the Flt4-L expression vector clone, and thus is biologically active. The sequence encoded by that open reading frame is shown in SEQ ID NO: 33. Plasmid pFLT4-L has been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852 as accession number 97231. The approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a nucleotide sequence that includes the 1997 nucleotides of sequence set forth in SEQ ID NO: 44. The nucleotide sequence set forth in SEQ ID NO: 44 encodes the 419 residue amino acid sequence set forth in SEQ ID NO: 45.

However, the predicted molecular weight of the mature protein product deduced from the reading frame specified in SEQ ID NOs: 32-33 is 35881 and the Flt4 ligand from PC-3 cell cultures had an approximate molecular weight of 23 kD under reducing conditions. It is thus possible that the Flt4-L mRNA may be first translated into a precursor, from which the mature ligand is derived by proteolytic cleavage. The difference in the observed molecular weight of the isolated Flt4 ligand and the deduced molecular weight of the disclosed open reading frame of the Flt4 ligand sequence may then derive from sequences in the carboxyl terminal region of the latter. Also, the Flt4 ligand may be glycosylated at two putative N-linked glycosylation sites, conforming to the consensus which can be identified in the deduced Flt4 ligand amino acid sequence (N-residues underlined in FIGS. 10B and 10C).

The carboxyl terminal amino acid sequences, which increase the predicted molecular weight of the Flt4 ligand

subunit in comparison with other ligands of this family, show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring protein 3 (BR3P) sequence (Dignam and Case, Gene 88, 133-140, 1990), as depicted in FIG. 9A. Such a sequence may encode an independently folded domain present in a Flt4 ligand precursor and it may be involved, for example, in the regulation of secretion, solubility, stability, cell surface localization or activity of the Flt4 ligand. Interestingly, at least one cysteine motif of the BR3P type is also found in the VEGF carboxy terminal amino acid sequences.

Thus, the Flt4-L mRNA may be first translated into a precursor from the mRNA corresponding to the Flt4-L clone, from which the mature ligand is derived by proteolytic cleavage. To define the mature Flt4 ligand product one first expresses the cDNA clone, which is deposited in the pcDNA1 expression vector, in cells, such as COS cells. One uses antibodies generated against Flt4-L-encoded peptides, such as amino terminal 23 amino acid peptide or bacterial Flt4 fusion proteins, such as a GST-fusion protein, to raise antibodies against the VEGF-homologous domain of Flt4 ligand. One then follows the biosynthesis and processing of the Flt4 ligand in the transfected cells by pulse-chase analysis using radioactive cysteine for labelling of the cells, immunoprecipitation and gel electrophoresis. Using antibodies against the two domains of the product of the Flt4-L clone material for radioactive or nonradioactive aminoterminal sequence analysis is isolated. The determination of the NH2-terminal sequence of the carboxyl terminal fragment allows for identification of the proteolytic processing site. This is confirmed by site-directed mutagenesis of the amino acid residues adjacent to the cleavage site, which would prevent the cleavage.

On the other hand, the Flt4 ligand is characterized by progressive 3' deletions in the 3' coding sequences of the Flt4 ligand precursor clone, resulting in carboxy-terminal truncations of its protein product. The activities of such truncated forms are assayed by, for example, studying Flt4 autophosphorylation induced by the truncated proteins when applied to cultures of cells, such as NIH3T3 cells expressing LTRFlt4. By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin et al., *Growth Factors* 8:245-252 (1993)) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues of the secreted VEGF-C protein lacking the signal sequence, and apparently within the first approximately 120 amino acid residues.

On the other hand, the difference between the molecular weights of the purified ligand and the open reading frame of the Flt4 precursor clone may be due to the fact that the soluble ligand was produced from an alternatively spliced mRNA which would also be present in the PC-3 cells, from which the isolated ligand was derived. To isolate such alternative cDNA clones one uses cDNA fragments of the deposited clone and PCR primers made according to the sequence provided as well as techniques standard in the art to isolate or amplify alternative cDNAs from the PC-3 cell cDNA library. One may also amplify using reverse transcription (RT)-PCR directly from the PC-3 mRNA using the primers provided in the sequence of the Flt4-L clone. Alternative cDNA sequences are determined from the resulting cDNA clones. One can also isolate genomic clones corresponding to the Flt4-L transcript from a human genomic DNA library using methods standard in the art and to sequence such clones or their subcloned fragments to reveal the corresponding exons. Alternative exons can then

recombinant VEGF-C stimulated tyrosine autophosphorylation of VEGFR-2 and the intensity of the autophosphorylated polypeptide band was increased upon concentration of the VEGF-C CM (lanes 3-5). Furthermore, the stimulating effect was abolished after pretreatment of the medium with the Flt4EC affinity matrix (compare lanes 1, 5 and 6). The maximal effect of VEGF-C in this assay was comparable to the effect of recombinant VEGF added to unconditioned medium at concentration of 50 ng/ml (lane 8). Pretreatment of the medium containing VEGF with Flt4EC did not abolish its stimulating effect on VEGFR-2 (compare lanes 7 and 8). These results suggest that the VEGF-C expression vector encodes a ligand not only for Flt4 (VEGFR-3), but also for VEGFR-2.

In order to further confirm that the stimulating effect of VEGF-C on tyrosine phosphorylation of VEGFR-3 and VEGFR-2 was receptor-specific, we analyzed the effect of VEGF-C on tyrosine phosphorylation of PDGF receptor β (PDGFR- β) which is abundantly expressed on fibroblastic cells. As can be seen from FIG. 14B, a weak tyrosine phosphorylation of PDGFR- β was detected upon stimulation of Flt4-expressing NIH3T3 cells with CM from the mock-transfected cells (compare lanes 1 and 2). A similar low level of PDGFR- β phosphorylation was observed when the cells were incubated with CM from the VEGF-C transfected cells, with or without prior treatment with Flt4EC (lanes 3 and 4). In contrast, the addition of 50 ng/ml of PDGF-BB induced a prominent tyrosine autophosphorylation of PDGFR- β (lane 5).

EXAMPLE 15

VEGF-C Stimulates Endothelial Cell Migration in Collagen Gel

CM from cell cultures transfected with the VEGF-C expression vector was placed in a well made in collagen gel and used to stimulate the migration of bovine capillary endothelial (BCE) cells in the three-dimensional collagen gel as follows.

BCE cells (Folkman et al., *Proc. Nat'l Acad. Sci. USA*, 76:5217-5221 (1979)) were cultured as described in (Pertovaara et al., *J. Biol. Chem.*, 269:6271-74 (1994)). The collagen gels were prepared by mixing type I collagen stock solution (5 mg/ml in 1 mM HCl) with an equal volume of 2xMEM and 2 volumes of MEM containing 10% newborn calf serum to give a final collagen concentration of 1.25 mg/ml. The tissue culture plates (5 cm diameter) were coated with about 1 mm thick layer of the solution, which was allowed to polymerize at 37°C. BCE cells were seeded on top of this layer. For the migration assays, the cells were allowed to attach inside a plastic ring (1 cm diameter) placed on top of the first collagen layer. After 30 min., the ring was removed and unattached cells were rinsed away. A second layer of collagen and a layer of growth medium (5% newborn calf serum (NCS)), solidified by 0.75% low melting point agar (FMC BioProducts, Rockland, Me.), were added. A well (3 mm diameter) was punched through all the layers on both sides of the cell spot at a distance of 4 mm, and the sample or control media were pipetted daily into the wells. Photomicrographs of the cells migrating out from the spot edge were taken after six days through an Olympus CK 2 inverted microscope equipped with phase-contrast optics. The migrating cells were counted after nuclear staining with the fluorescent dye bisbenzimidazole (1 mg/ml, Hoechst 33258, Sigma).

FIG. 15A depicts a comparison of the number of cells migrating at different distances from the original area of

attachment towards wells containing media conditioned by the non-transfected (control) or transfected (mock: VEGF-C: VEGF) cells, 6 days after addition of the media. The number of cells migrating out from the original ring of attachment was counted in five adjacent 0.5 mmx0.5 mm squares using a microscope ocular lens grid and 10x magnification. Cells migrating further than 0.5 mm were counted in a similar way by moving the grid in 0.5 mm steps. The experiments were carried out twice with similar results, and medium values from the one of the experiments are presented with standard error bars. The photographs in FIG. 15B depict phase-contrast microscopy and fluorescent microscopy of the nuclear staining of BCE cells migrating towards the wells containing media conditioned by the mock-transfected cells or by VEGF-C-transfected cells. The areas shown is approximately 1 mmx1.5 mm, and arrows indicate the borders of the original ring of attachment.

After 6 days of treatment, the cultures were stained and cells at different distances outside of the original ring of attachment were counted using fluorescent nuclear staining and detection with a fluorescence microscope equipped with a grid. A comparison of the numbers of migrating cells in successive 0.5 mmx0.5 mm areas is shown in FIG. 15A. As can be seen from the columns, VEGF-C-containing CM stimulated cell migration more than medium conditioned by the non-transfected or mock-transfected cells but less than medium from cells transfected with a VEGF expression vector. An example of typical phase contrast and fluorescent microscopic fields of cultures stimulated with medium from mock-transfected or VEGF-C transfected cells is shown in FIG. 15B. Daily addition of 1 ng of FGF2 into the wells resulted in the migration of approximately twice the number of cells when compared to the stimulation by CM from VEGF-transfected cells.

EXAMPLE 16

VEGF-C is Expressed in Multiple Tissues

Northern blots containing 2 micrograms of isolated poly (A)⁺ RNA from multiple human tissues (blot from Clontech) were probed with radioactively labelled insert of the 2.0 kb VEGF-C cDNA clone. Northern blotting and hybridization analysis showed that the 2.4 kb RNA and smaller amounts of a 2.0 kb mRNA are expressed in multiple human tissues, most prominently in the heart, placenta, muscle, ovary and small intestine (FIG. 16A). Very little VEGF-C RNA was seen in the brain, liver or thymus and peripheral blood leukocytes (pbl) appeared negative. A similar analysis of RNA from human fetal tissues (FIG. 16B) shows that VEGF-C is highly expressed in the kidney and lung and to a lesser degree in the liver, while essentially no expression is detected in the brain. Interestingly, VEGF expression correlates with VEGF-C expression in these tissues, whereas VEGF-B is highly expressed in all tissues analyzed.

EXAMPLE 17

The VEGF-C Gene Localizes to Chromosome 4q34

A DNA panel of 24 interspecies somatic cell hybrids, which had retained one or two human chromosomes, was used for the chromosomal localization of the VEGF-C gene (Bios Laboratories, Inc., New Haven, Conn.). Primers were designed to amplify an about 250 bp fragment of the VEGF-C gene from somatic cell hybrid DNA. The primers and conditions for polymerase chain reaction (PCR) were

5'-TGAGTGATTGTAGCTGTGTG-3' (forward) [SEQ ID NO:34] and 5'-TATTGCAGCAACCCACATCT-3' (reverse) [SEQ ID NO:35] for VEGF-C (94° C., 60s/62° C., 45s/72° C., 60s). The PCR products were evaluated by electrophoresis in 1% agarose gels and visualized by ethidium bromide staining in ultraviolet light. [α -³²P]-dCTP-labelled cDNA inserts of a plasmid representing the complete VEGF-C coding domain was used as a probe in Southern blotting and hybridization analysis of the somatic cell hybrid DNAs as instructed by the supplier (Bios Laboratories).

The cell lines for fluorescence in situ hybridization (FISH) were obtained from the American Type Culture Collection (Rockville, Md.). Purified DNA from P1 clones 7660 and 7661 (VEGF-C) (Genome Systems, Inc., St. Louis, Mo.) were confirmed positive by Southern blotting of Eco RI-digested DNA followed by hybridization with the VEGF-C cDNA. The P1 clones were then labelled by nick translation either with biotin-11-dUTP, biotin-14-ATP (Sigma Chemical Co., St. Louis, Mo.) or digoxigenin 11-dUTP (Boehringer Mannheim GmbH, Mannheim, Germany) according to standard protocols. PHA-stimulated peripheral blood lymphocyte cultures were treated with 5-bromodeoxyuridine (BrdU) at an early replicating phase to induce G-banding. See Takahashi et al., *Human Genet.*, 86:14-16 (1995); Lemieux et al., *Cytogenet. Cell Genet.*, 59:311-12 (1992). The FISH procedure was carried out in 50% formamide, 10% dextran sulphate in 2xSSC using well-known procedures. See, e.g., Rytönen et al., *Cytogenet. Cell Genet.*, 68:61-63 (1995); Lichter et al., *Proc. Natl. Acad. Sci. USA*, 85:9664-68 (1988). Repetitive sequences were suppressed with 50-fold excess of Cot-1 DNA (BRL, Gaithersburg, Md.) compared with the labeled probe. Specific hybridization signals were detected by incubating the hybridized slides in labelled antidigoxigenin antibodies, followed by counterstaining with 0.1 mmol/L 4,6-diamino-2-phenylindole. Probe detection for two-color experiments was accomplished by incubating the slides in fluorescein isothiocyanate (FITC)-anti-digoxigenin antibodies (Sigma Chemical Co.) and Texas red-avidin (Vector Laboratories, Burlingame, Calif.) or rhodamine-anti-digoxigenin and FITC-avidin.

Multi-color digital image analysis was used for acquisition, display and quantification of hybridization signals of metaphase chromosomes. The system contains a PXL camera (Photometrics Inc., Tucson, Ariz.) attached to a PowerMac 7100/AV workstation. IPLab software controls the camera operation, image acquisition and Ludl Filter wheel. At least 50 nuclei were scored. Overlapping nuclei and clusters of cells were ignored. A slide containing normal lymphocyte metaphase spreads and interphase nuclei was included in each experiment to control for the efficiency and specificity of the hybridization.

In order to determine the chromosomal localization of the human VEGF-C gene, DNAs from human rodent somatic cell hybrids containing defined sets of human chromosomes were analyzed by Southern blotting and hybridization with the VEGF-C cDNA probe. Among 24 DNA samples on the hybrid panel, representing different human chromosomes, human-specific signals were observed only in hybrids which contained human chromosome 4. The results were confirmed by PCR of somatic cell hybrid DNA using VEGF-C specific primers, where amplified bands were obtained only from DNAs containing human chromosome 4.

A genomic P1 plasmid for VEGF-C was isolated using specific primers and PCR and verified by Southern blotting and hybridization using a VEGF-C specific cDNA probe.

The chromosomal localization of VEGF-C was further studied using metaphase FISH. Using the P1 probe for VEGF-C in FISH a specific hybridization to the 4q34 chromosomal band was detected in 40 out of 44 metaphases (FIG. 17). Double-fluorochrome hybridization using a cosmid probe specific for the aspartylglucosaminidase (AGA) gene showed that VEGF-C is located just proximal to the AGA gene previously mapped to the 4q34-35 chromosomal band.

Biotin labelled VEGF-C P1 and digoxigenin labeled AGA cosmid probes were hybridized simultaneously to metaphase chromosomes. This experiment demonstrated that the AGA gene is more telomerically located than the VEGF-C gene. The foregoing example demonstrates the utility of polynucleotides of the invention as chromosomal markers.

EXAMPLE 18

Effect of Glucose Concentration and Hypoxia on VEGF, VEGF-B and VEGF-C mRNA levels in C6 Glioblastoma Cells

Confluent cultures of C6 cells (ATCC CCL 107) were grown on 10 cm diameter tissue culture plates containing 2.5 ml of DMEM and 5% fetal calf serum plus antibiotics. The cultures were exposed for 16 hours to normoxia in a normal cell culture incubator containing 5% CO₂ (FIG. 18: lanes marked -) or hypoxia (FIG. 18: lanes marked +) by closing the culture plates in an airtight glass chamber and burning a piece of wood inside until the flame was extinguished due to lack of oxygen. Polyadenylated RNA was isolated (as in the other examples), and 8 micrograms of the RNA was electrophoresed and blot-hybridized with a mixture of the VEGF, VEGF-B and VEGF-C probes (see FIG. 12). The results show that hypoxia strongly induces VEGF-A mRNA expression (compare lanes - and +), both in low and high glucose, but has no significant effect on the VEGF-B mRNA levels. The VEGF-C mRNA isolated from hypoxic cells runs slightly faster in gel electrophoresis and an extra band of faster mobility can be seen below the upper mRNA band. This observation suggests that hypoxia affects VEGF-C RNA processing. One explanation for this observation is that VEGF-C mRNA splicing is altered, affecting the VEGF-C open reading frame and resulting in an alternative VEGF-C protein being produced by hypoxic cells. Such alternative forms of VEGF-C and VEGF-C-encoding polynucleotides are contemplated as an aspect of the invention.

Deposit of Biological Materials: Plasmid FLT4-L has been deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville Md. 20852 (USA), pursuant to the provisions of the Budapest Treaty, and has been assigned a deposit date of Jul. 24, 1995 and ATCC accession number 97231.

While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those in the art. Accordingly, only such limitations as appear in the appended claims should be placed on the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 45

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTCCTCGCT GTCTTGTCT

20

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACATGCATGC CACCATGCAG CCGGGCGCGC CGCTGTGCTT GCGACTGTGG CTCTGCGTGG

50

GACTCCTGGA

70

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACATGCATGC CCGCGCGGTC ATCC

24

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGAATTCCC CATGACCCCA AC

22

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
 CCATCGATGG ATCTACCTG AAGCCGCTTT CTT 33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
 ATTTAGGTGA CACTATA 17

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
 CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT 34

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 40 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
 Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
 1 5 10 15
 Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
 20 25 30
 His Arg Gln Glu Ser Gly Phe Arg
 35 40

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
 CTGGAGTCCA CTGGCGGAC T 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 60 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single

-continued

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGGTGC CTTATCCTC 60

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC 34

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTTGCCTGTG ATGTGACCA 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
 1 5 10 15
 Leu Lys

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCAGARGARA CHATHAA 17

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Glu Thr Ile Lys
1 5

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAYTTNARD ATYTONGT

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Thr Glu Ile Leu Lys
1 5

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTCGCTGCA GCACACTACA AC

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:19:

DISCUSSION

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(11) MOLECULE TYPE: peptide

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Ala His Tyr Asn Thr Glu
1 5

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TAATACGACT CACTATAGG

20

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Phe Ala Ala Ala His Tyr Asn
1 5

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCACTATAGG GAGACCCAG C

21

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 219 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
GTTGTAGTGT GCTGCAGCGA ATTT 24

(2) INFORMATION FOR SEQ ID NO:31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
TCACTATAGG GAGACCCAAG C 21

(2) INFORMATION FOR SEQ ID NO:32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1140 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 37..1086
(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 136..1086
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAATC ATG ACT GTA CTC TAC CCA 54
Met Thr Val Leu Tyr Pro
-33 -30
GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA 102
Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln
-25 -20 -15
CAT AAC ACA GAA CAG GGC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA 150
His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile
-10 -5 1 5
AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT 198
Lys Phe Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp
10 15 20
AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT 246
Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp
25 30 35
GTC GGC AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA 294
Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro
40 45 50
TGT GTG TCC GTC TAC AGA TGT GCG GGT TGC TGC AAT AGT GAG GGG CTG 342
Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu
55 60 65
CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA 390
Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu
70 75 80 85
ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT 438
Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe
90 95 100
GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA 486
Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg

-continued

105	110	115	
CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG			534
Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln			
120	125	130	
TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT			582
Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn			
135	140	145	
CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT			630
His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp			
150	155	160	165
GCT GGA CAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC			678
Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn			
170	175	180	
AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT			726
Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu			
185	190	195	
CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC			774
Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys			
200	205	210	
CAG TGT GTC TGT AAA AAC AAA CTC TTC CCC AGC CAA TGT GGG GCC AAC			822
Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn			
215	220	225	
CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC			870
Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys			
230	235	240	245
CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA			918
Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr			
250	255	260	
GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA			966
Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln			
265	270	275	
ACA TGC AGC TGT TAC AGA CCG CCA TGT ACG AAC CCG CAG AAG GCT TGT			1014
Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys			
280	285	290	
GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA			1062
Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser			
295	300	305	
TAT TGG AAA AGA CCA CAA ATG AGC TAAGATTGTA CTGTTTCCA GTTCATCGAT			1116
Tyr Trp Lys Arg Pro Gln Met Ser			
310	315		
TTTCTATTAT GGAAACTGT GTTG			1140

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 350 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu
 -33 -30 -25 -20

Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser
 -15 -10 -5

Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu
 1 5 10 15

Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro
 20 25 30

-continued

Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn
 35 40 45
 Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys
 50 55 60
 Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu
 65 70 75
 Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys
 80 85 90 95
 Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser
 100 105 110
 Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu
 115 120 125
 Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr
 130 135 140
 Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp
 145 150 155
 Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
 160 165 170 175
 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Thr Cys Gln Cys
 180 185 190
 Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu
 195 200 205
 Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro
 210 215 220
 Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys
 225 230 235
 Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys
 240 245 250 255
 Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly
 260 265 270
 Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr
 275 280 285
 Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val
 290 295 300
 Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser
 305 310 315

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TGAGTGATTGTAGCTGCTGTG

22

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

-continued

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TATTGCAGCAACCCACATCT

22

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 196 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```

Met Arg Thr Trp Ala Cys Leu Leu Leu Leu Gly Cys Gly Tyr Leu Ala
1      5      10      15
His Ala Leu Ala Glu Glu Ala Glu Ile Pro Arg Glu Leu Ile Glu Arg
20     25     30
Leu Ala Arg Ser Gln Ile His Ser Ile Arg Asp Leu Gln Arg Leu Leu
35     40     45
Glu Ile Asp Ser Val Gly Ala Glu Asp Ala Leu Glu Thr Ser Leu Arg
50     55     60
Ala His Gly Ser His Ala Ile Asn His Val Pro Glu Lys Arg Pro Val
65     70     75     80
Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Ile Pro Ala Val Cys
85     90     95
Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro
100    105    110
Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg
115    120    125
Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg
130    135    140
Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys
145    150    155    160
Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu
165    170    175
Cys Ala Cys Ala Thr Ser Asn Leu Asn Pro Asp His Arg Glu Glu Glu
180    185    190
Thr Asp Val Arg
195

```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 241 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```

Met Asn Arg Cys Trp Ala Leu Phe Leu Ser Leu Cys Cys Tyr Leu Arg
1      5      10      15
Leu Val Ser Ala Glu Gly Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met
20     25     30
Leu Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu
35     40     45

```

-continued

His Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met
 50 55 60
 Thr Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Arg
 65 70 75 80
 Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu
 85 90 95
 Cys Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp
 100 105 110
 Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln
 115 120 125
 Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr
 130 135 140
 Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg
 145 150 155 160
 Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu
 165 170 175
 Ala Cys Lys Cys Glu Thr Val Ala Ala Arg Pro Val Thr Arg Ser
 180 185 190
 Pro Gly Gly Ser Gln Glu Gln Arg Ala Lys Thr Pro Gln Thr Arg Val
 195 200 205
 Thr Ile Arg Thr Val Arg Val Arg Arg Pro Pro Lys Gly Lys His Arg
 210 215 220
 Lys Phe Lys His Thr His Asp Lys Thr Ala Leu Lys Glu Thr Leu Gly
 225 230 235 240
 Ala

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 149 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: Not Relevant
 (D) TOPOLOGY: Not Relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
 1 5 10 15
 Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
 20 25 30
 Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
 35 40 45
 Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
 50 55 60
 Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
 65 70 75 80
 Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
 85 90 95
 Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
 100 105 110
 Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
 115 120 125
 Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Cys Gly Asp
 130 135 140

-continued

Ala Val Pro Arg Arg
145

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 170 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1           5           10           15

Leu Ala Leu Pro Ala Val Pro Pro Gln Trp Ala Leu Ser Ala Gly
20           25           30

Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
35           40           45

Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
50           55           60

Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65           70           75           80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
85           90           95

Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
100          105          110

Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
115          120          125

Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Arg Arg Pro
130          135          140

Lys Gly Arg Gly Lys Arg Arg Glu Lys Gln Arg Pro Thr Asp Cys
145          150          155          160

His Leu Cys Gly Asp Ala Val Pro Arg Arg
165          170

```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 147 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1           5           10           15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20           25           30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35           40           45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50           55           60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65           70           75           80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85           90           95

```

-continued

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Cys Asp Lys
130 135 140
Pro Arg Arg
145

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: Not Relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
130 135 140
Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145 150 155 160
Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
165 170 175
Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
180 185 190

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 215 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: Not Relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly

-continued

20	25	30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln		
35	40	45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu		
50	55	60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu		
65	70	75
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro		
85	90	95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His		
100	105	110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys		
115	120	125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val		
130	135	140
Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr		
145	150	155
Lys Ser Trp Ser Val Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His		
165	170	175
Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr		
180	185	190
Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys		
195	200	205
Arg Cys Asp Lys Pro Arg Arg		
210	215	

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 232 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Relevant
- (D) TOPOLOGY: Not Relevant

(ii) MOLECULE TYPE: protein

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu		
1	5	10
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly		
20	25	30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln		
35	40	45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu		
50	55	60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu		
65	70	75
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro		
85	90	95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His		
100	105	110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys		
115	120	125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val		
130	135	140
Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr		

-continued

145	150	155	160
Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys Leu Met Pro Trp			
	165	170	175
Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys			
	180	185	190
His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn			
	195	200	205
Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr			
	210	215	220
Cys Arg Cys Asp Lys Pro Arg Arg			
225	230		

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1997 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```

CCCCCCCCCG CTCACAAA AGCTACACCG ACGCGGACCG CGCGGCGCTC CTCCTCGCC      60
CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GAGCTCGGA TGTCCGTTT CCGTGAGGC      120
TTTACCTGA CACCGCGCGC CTTCCTCGCG CACTGCTGG GAGGCGCCCC TGCAAGTTG      180
GGAACGCGGA GCGCGGACCT CGCTCCCGCC GCTCCCGCT CGCCAGGGG GGGTCGCGCG      240
GAGGAGCGCG GGGGAGAGGG ACCAGGAGGG GCGCGCGGCC TCGAGGGGG GCGCGCGGCC      300
CCACCCCTGC CCGCGGACG GAGCGGTCC CCCACCCCG GTCTTCAC C ATG CAC      357
Met His
1
TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG      405
Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu
5 10 15
CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC TTC GAG TCC      453
Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe Glu Ser
20 25 30
GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT      501
Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala
35 40 45 50
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA      549
Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val
55 60 65
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG      597
Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys
70 75 80
TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC      645
Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn
85 90 95
CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT      693
Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr
100 105 110
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA      741
Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln
115 120 125 130

```

-continued

TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GCG AAG CAG TTT CGA GTC Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val	789
135 140 145	
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys	837
150 155 160	
GGG GGT TGC TGC AAT AGT GAG GCG CTG CAG TGC ATG AAC ACC AGC ACG Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr	885
165 170 175	
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln	933
180 185 190	
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg	981
195 200 205 210	
TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg	1029
215 220 225	
CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr	1077
230 235 240	
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala	1125
245 250 255	
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp	1173
260 265 270	
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr	1221
275 280 285 290	
TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro	1269
295 300 305	
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys	1317
310 315 320	
CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr	1365
325 330 335	
TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn	1413
340 345 350	
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu	1461
355 360 365 370	
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CCG Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg	1509
375 380 385	
CCA TGT ACG AAC CCG CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser	1557
390 395 400	
GAA GAA GTG TGT GGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met	1605
405 410 415	
AGC TAAGATTGTA CTGTTTCCA GTTCATCGAT TTCTATTAT GGAAACTGT Ser	1658
GTGCGACAG TAGAAGTGTG TGTGAACAGA GAGACCTTG TGGGTCCATG CTACAAAGA	1718
CAAAAGTGTG TCTTCTGTA ACCATGTGGA TAACCTTACA GAAATGGACT GGAGCTCATC	1778

-continued

TGCAGAGGC CTCTGTAAA GACTGGTTT CTGCCAATGA CCAACAGCC AAGATTTTC 1838
 TCTGTGATT TCTTAAAG AATGACTATA TAATTTATT CCACTAAAA TATTGTTCT 1898
 GCATTCAATT TTATAGCAAC AACAAITGGT AAAACTCACT GTGATCAATA TTTTATATC 1958
 ATGCAAAATA TGTTTAAAT AAAATGAAA TTGTATTAT 1997

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 419 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
 1 5 10 15
 Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe
 20 25 30
 Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
 35 40 45
 Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
 50 55 60
 Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
 65 70 75 80
 Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
 85 90 95
 Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
 100 105 110
 His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
 115 120 125
 Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
 130 135 140
 Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
 145 150 155 160
 Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
 165 170 175
 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
 180 185 190
 Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
 195 200 205
 Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
 210 215 220
 Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
 225 230 235 240
 Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
 245 250 255
 Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
 260 265 270
 Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
 275 280 285
 Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
 290 295 300
 Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys

-continued

305	310	315	320
Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu			
325	330	335	
Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro			
340	345	350	
Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys			
355	360	365	
Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr			
370	375	380	
Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser			
385	390	395	400
Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro			
405	410	415	
Gln Met Ser			

What is claimed is:

1. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds to human Flt4 receptor tyrosine kinase (Flt4), said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.
2. A purified and isolated nucleic acid according to claim 1 wherein said polypeptide stimulates tyrosine phosphorylation of human Flt4.
3. A purified and isolated nucleic acid according to claim 2 wherein said polypeptide has an apparent molecular weight of about 23 kD as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.
4. A purified and isolated nucleic acid according to claim 2 wherein said polypeptide comprises an amino-terminal amino acid sequence set forth in SEQ ID NO: 13.
5. A purified and isolated nucleic acid according to claim 4 wherein said polypeptide comprises approximately 120 amino acids.
6. A purified and isolated nucleic acid according to claim 4 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence identical to amino acids 2 through 18 set forth in SEQ ID NO: 13.
7. A purified and isolated nucleic acid according to claim 2 wherein said polypeptide comprises amino acids 1 to 120 of SEQ ID NO: 33.
8. A purified and isolated nucleic acid according to claim 1 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.
9. A nucleic acid according to claim 1 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes eight cysteines or SEQ ID NO: 33 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and excludes the carboxyl terminal portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.
10. A nucleic acid according to claim 9 wherein said continuous portion has amino acid 1 of SEQ ID NO: 33 as its amino terminus.
11. A nucleic acid according to claim 1 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.
12. A vector comprising a nucleic acid according to claim 1, wherein said vector lacks a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.
13. A host cell transformed or transfected with a vector according to claim 12.
14. A method for producing a polypeptide that binds to the extracellular domain of human Flt4, comprising the steps of: growing a host cell according to claim 13 under conditions which permit expression by said host cell of a polypeptide that is encoded by said nucleic acid and that binds to the extracellular domain of human Flt4; and isolating said polypeptide from the host cell or the growth medium of the host cell.
15. A method according to claim 14 wherein said host cell is a mammalian host cell that secretes said polypeptide and wherein said isolating step comprises isolating said polypeptide from said growth medium.
16. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds human Flt4 receptor tyrosine kinase (Flt4), said polypeptide having an amino acid sequence comprising a continuous portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.
17. A purified and isolated nucleic acid according to claim 16 wherein said encoded polypeptide stimulates tyrosine phosphorylation of human Flt4.
18. A purified and isolated nucleic acid according to claim 16 wherein said nucleic acid lacks a nucleotide sequence that encodes the amino terminal portion of the amino acid sequence shown in SEQ ID NO: 33 that precedes residue 1.
19. An expression construct comprising the nucleic acid according to claim 18 operatively linked to an expression control sequence, said expression construct lacking a nucle-

otide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO:33 beyond residue 125.

20. A host cell transformed or transfected with the expression construct of claim 19.

21. A method for producing a polypeptide that binds to the extracellular domain of human Flt4 and stimulates tyrosine phosphorylation of Flt4, comprising the steps of:

- growing a host cell according to claim 20 under conditions which permit expression in said host cell of a polypeptide encoded by said nucleic acid and
- isolating said polypeptide from the host cell or the growth medium of the host cell, wherein said polypeptide binds to the extracellular domain of human Flt4 and stimulates phosphorylation of Flt4.

22. A host cell transformed or transfected with a polynucleotide,

wherein said polynucleotide includes a strand containing a human nucleotide sequence that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

- (a) hybridization at 42° C. for 20 hours in a solution containing 50% formamide, 5xSSPE, 5xDenhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and
- (b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65° C. with a wash solution containing 1xSSC, and 0.1% SDS; and

wherein said host cell expresses a polypeptide encoded by said polynucleotide,

wherein said polypeptide has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions, and includes a domain encoded by the human nucleotide sequence that is defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B),

wherein said polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide binds to the extracellular domain of human Flt4 receptor tyrosine kinase.

23. A host cell according to claim 22 that expresses a naturally occurring human Flt4 ligand polypeptide encoded by said polynucleotide.

24. A host cell according to claim 22 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to sequence that encodes said polypeptide.

25. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a contiguous portion of SEQ ID NO: 33 that is sufficient to bind to the extracellular domain of human Flt4 receptor tyrosine kinase (Flt4EC),

wherein said contiguous portion includes eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B),

wherein said polypeptide lacks any portion of SEQ ID NO: 33 that precedes position 1 and lacks any portion

of SEQ ID NO: 33 that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide has a molecular weight of about 23 kD as assessed by SDS PAGE under reducing conditions and binds to Flt4EC.

26. A host cell according to claim 25 wherein said nucleotide sequence comprises nucleotides 37 to 1086 of the sequence shown in SEQ ID NO: 32.

27. A host cell according to claim 25 wherein said polynucleotide is a vector comprising an expression control sequence operatively linked to the nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO: 33.

28. A eukaryotic host cell according to claim 22 or 25 that secretes said polypeptide.

29. A host cell comprising the insert of plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses and secretes a polypeptide encoded by said insert,

wherein said secreted polypeptide has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions and binds to human Flt4 receptor tyrosine kinase and includes a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).

30. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that binds to the extracellular domain of human Flt4 receptor tyrosine kinase,

wherein said polynucleotide includes a strand containing a human nucleotide sequence that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

- (a) hybridization at 42° C. for 20 hours in a solution containing 50% formamide, 5xSSPE, 5xDenhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and
- (b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65° C. with a wash solution containing 1xSSC, and 0.1% SDS; and

wherein said host cell expresses and secretes a polypeptide encoded by said polynucleotide, and

wherein said expressed and secreted polypeptide binds the extracellular domain of human Flt4 receptor tyrosine kinase and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

31. A method for producing a polypeptide that binds the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

- growing a host cell according to any one of claims 21-27,
- 29, or 30 under conditions which permit expression by said host cell of said polypeptide; and
- isolating said polypeptide from the host cell or the growth medium of the host cell.

32. A method for producing a polypeptide that binds to the extracellular domain (EC) of human Flt4 receptor tyrosine kinase (Flt4), comprising steps of:

- growing a host cell comprising a polynucleotide that comprises a nucleotide sequence that encodes the amino acid sequence set forth in SEQ ID NO:33, under conditions in which the host cell expresses and secretes a polypeptide encoded by the polynucleotide; and
- isolating a polypeptide that binds Flt4 EC from the growth medium of the host cell, said polypeptide having a

65

molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions and having an amino acid sequence comprising a portion of SEQ ID NO:33 effective to bind Flt4 EC.

33. A method according to claim 32 wherein said polynucleotide comprises an expression vector that comprises a nucleotide sequence that encodes the amino acid set forth in SEQ ID NO:33.

66

34. A method according to claim 32 wherein said host cell comprises a PC-3 prostatic adenocarcinoma cell (ATCC CRL1435).

35. A method according to claim 32 wherein said polynucleotide comprises the insert of plasma pFLT4-L, deposited as ATCC Accession No. 97231.

* * * * *





00 58589

PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:)	"EXPRESS MAIL"
Alitalo et al.)	Mailing label No. EG473137204US
Serial No.: Not yet assigned)	Date of Deposit: January 12, 1996
Filed: Herewith)	I hereby certify that this paper (or fee) is
For: Receptor Ligand)	being deposited with the United States
Group Art Unit: Not yet assigned)	Postal Service "EXPRESS MAIL POST
Examiner: Not yet assigned)	OFFICE TO ADDRESSEE" service under
)	37 CFR §1.10 on the date indicated above
)	and is addressed to the Assistant
)	Commissioner for Patents,
)	Washington, D.C., 20231.
)	<i>David A. Gass</i>
)	David A. Gass

STATEMENT PURSUANT TO 37 C.F.R. §1.821(f)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby state that the content of the paper and computer readable forms of the sequence listing that is part of the above-identified application and that are filed herewith are the same.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Dated: January 12, 1996

David A. Gass
David A. Gass
Registration No. 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, IL 60606-6402
Telephone: (312) 474-6300

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 1995

Application or Docket Number

08/585895

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
FOR	NUMBER FILED	NUMBER EXTRA
BASIC FEE		
TOTAL CLAIMS	16 minus 20 =	*
INDEPENDENT CLAIMS	3 minus 3 =	*
MULTIPLE DEPENDENT CLAIM PRESENT		

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
	375.00	OR		750.00
x\$11=		OR	x\$22=	
x39=		OR	x78=	
+125=		OR	+250=	
TOTAL		OR	TOTAL	750-

CLAIMS AS AMENDED - PART II

	(Column 1)	(Column 2)	(Column 3)
	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	23 Minus	20	= 13
Independent	3 Minus	3	= 0
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=	\$143 ⁰⁰	OR	x\$22=	
x39=	\$82 ⁰⁰	OR	x78=	
+125=	\$135 ⁰⁰	OR	+250=	
TOTAL ADDIT. FEE	\$360 ⁰⁰	OR	TOTAL ADDIT. FEE	

	(Column 1)	(Column 2)	(Column 3)
	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	Minus	37	=
Independent	Minus	5	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x39=		OR	x78=	
+125=		OR	+250=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

	(Column 1)	(Column 2)	(Column 3)
	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	35 Minus	37	=
Independent	18 Minus	5	= 2
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x39=	78	OR	x78=	
+125=	C	OR	+250=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
 ** If the "Highest Number Previously Paid For" in THIS SPACE is less than 20, enter "20."
 *** If the "Highest Number Previously Paid For" in THIS SPACE is less than 3, enter "3."
 The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

FROM: George M. Rayford, Manager
Correspondence & Mail Division

SUBJECT: Receipt of Papers and Fees File Under 37 CFR 1.10 By
Express Mail

The filing date of _____ is the correct date. The date on the Express Mail label under 37 CFR 1.10 is _____. On that date the PTO was closed all day due to _____ adverse weather conditions (authorized by Office of Personnel Management) or a _____ normally scheduled Federal holiday within the District of Columbia. In accordance with 37 CFR 1.6 the papers have been stamped with the next succeeding day which is not a Saturday, Sunday or Federal holiday within the District of Columbia. The provision of 35 U.S.C. 21 (b) apply.

The papers were not stamped with the date on the certificate of mailing by Express Mail because the date on the certificate does not coincide with the date of deposit on the Express Mail label which the PTO takes as evidence of when the package was mailed.

Date on certificate of mailing by Express Mail is 1-12-96

Date on Express Mail label is 1-12-96

Date of receipt in PTO is 1-16-96

Therefore, the filing date is 1-16-96

The papers are not entitled to the benefits of 37 CFR 1.10 because:

You are unable to receive the date on your
mail certificate or mailing label because of
the inclement weather conditions therefore you will
receive the date the PTO receive it which is 1-16-96

SIGNED: Julia H. Woodford

DATE: 1-19-96

PAGE: 1

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION US/08/585,895

DATE: 04/11/96
TIME: 14:24:34

INPUT SET: S9820.raw

Line

Error

Original Text

PAGE: 5

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895

DATE: 04/11/96
TIME: 14:24:31

INPUT SET: S9820.raw

206 (i) SEQUENCE CHARACTERISTICS:
207 (A) LENGTH: 20 base pairs
208 (B) TYPE: nucleic acid
209 (C) STRANDEDNESS: single
210 (D) TOPOLOGY: linear

211
212 (ii) MOLECULE TYPE: DNA (genomic)

213
214 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

215
216 GTTGCCTGTG ATGTGCACCA

20

217
218 (2) INFORMATION FOR SEQ ID NO:13:

219 (i) SEQUENCE CHARACTERISTICS:
220 (A) LENGTH: 18 amino acids
221 (B) TYPE: amino acid
222 (C) STRANDEDNESS: single
223 (D) TOPOLOGY: linear

224
225 (ii) MOLECULE TYPE: peptide

226
227 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

228 Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
229 1 5 10 15

230
231 Leu Lys

232
233 (2) INFORMATION FOR SEQ ID NO:14:

234 (i) SEQUENCE CHARACTERISTICS:
235 (A) LENGTH: 17 base pairs
236 (B) TYPE: nucleic acid
237 (C) STRANDEDNESS: single
238 (D) TOPOLOGY: linear

239
240 (ii) MOLECULE TYPE: DNA (genomic)

241
242 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

243
244 GCACARGARA CNATHAA

17

245
246 (2) INFORMATION FOR SEQ ID NO:15:

247 (i) SEQUENCE CHARACTERISTICS:
248 (A) LENGTH: 5 amino acids
249 (B) TYPE: amino acid
250 (C) STRANDEDNESS: single
251 (D) TOPOLOGY: linear

252
253 (ii) MOLECULE TYPE: peptide

PAGE: 4

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895

DATE: 04/11/96
TIME: 14:24:27

INPUT SET: S9820.raw

```
153      Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
154      1              5              10              15
155
156      Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
157      20              25              30
158
159      His Arg Gln Glu Ser Gly Phe Arg
160      35              40
161
162      (2) INFORMATION FOR SEQ ID NO:9:
163
164          (i) SEQUENCE CHARACTERISTICS:
165              (A) LENGTH: 21 base pairs
166              (B) TYPE: nucleic acid
167              (C) STRANDEDNESS: single
168              (D) TOPOLOGY: linear
169
170          (ii) MOLECULE TYPE: DNA (genomic)
171
172          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
173
174      CTGGAGTCGA CTTGGCGGAC T
175
176      (2) INFORMATION FOR SEQ ID NO:10:
177
178          (i) SEQUENCE CHARACTERISTICS:
179              (A) LENGTH: 60 base pairs
180              (B) TYPE: nucleic acid
181              (C) STRANDEDNESS: single
182              (D) TOPOLOGY: linear
183
184          (ii) MOLECULE TYPE: DNA (genomic)
185
186          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
187
188      CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CTTATCCTC
189
190      (2) INFORMATION FOR SEQ ID NO:11:
191
192          (i) SEQUENCE CHARACTERISTICS:
193              (A) LENGTH: 34 base pairs
194              (B) TYPE: nucleic acid
195              (C) STRANDEDNESS: single
196              (D) TOPOLOGY: linear
197
198          (ii) MOLECULE TYPE: DNA (genomic)
199
200          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
201
202      CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC
203
204      (2) INFORMATION FOR SEQ ID NO:12:
205
```

21

60

34

PAGE: 3

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895

DATE: 04/11/96
TIME: 14:24:24

INPUT SET: S9820.raw

100
101 (1) SEQUENCE CHARACTERISTICS:
102 (A) LENGTH: 33 base pairs
103 (B) TYPE: nucleic acid
104 (C) STRANDEDNESS: single
105 (D) TOPOLOGY: linear
106
107 (ii) MOLECULE TYPE: DNA (genomic)
108
109 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
110
111 CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT
112
113 (2) INFORMATION FOR SEQ ID NO:6:
114
115 (1) SEQUENCE CHARACTERISTICS:
116 (A) LENGTH: 17 base pairs
117 (B) TYPE: nucleic acid
118 (C) STRANDEDNESS: single
119 (D) TOPOLOGY: linear
120
121 (ii) MOLECULE TYPE: DNA (genomic)
122
123 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
124
125 ATTTAGGTGA CACTATA
126
127 (2) INFORMATION FOR SEQ ID NO:7:
128
129 (1) SEQUENCE CHARACTERISTICS:
130 (A) LENGTH: 34 base pairs
131 (B) TYPE: nucleic acid
132 (C) STRANDEDNESS: single
133 (D) TOPOLOGY: linear
134
135 (ii) MOLECULE TYPE: DNA (genomic)
136
137 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
138
139 CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT
140
141 (2) INFORMATION FOR SEQ ID NO:8:
142
143 (1) SEQUENCE CHARACTERISTICS:
144 (A) LENGTH: 40 amino acids
145 (B) TYPE: amino acid
146 (C) STRANDEDNESS: single
147 (D) TOPOLOGY: linear
148
149 (ii) MOLECULE TYPE: protein
150
151 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
152

33

17

34

PAGE: 2

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895

DATE: 04/11/96
TIME: 14:24:20

INPUT SET: S9820.raw

```

47         (D) TOPOLOGY: linear
48
49     (ii) MOLECULE TYPE: DNA (genomic)
50
51     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
52
53     TGTCTCGCT GTCCTTGTCT
54
55     (2) INFORMATION FOR SEQ ID NO:2:
56
57     (i) SEQUENCE CHARACTERISTICS:
58         (A) LENGTH: 70 base pairs
59         (B) TYPE: nucleic acid
60         (C) STRANDEDNESS: single
61         (D) TOPOLOGY: linear
62
63     (ii) MOLECULE TYPE: DNA (genomic)
64
65     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
66
67     ACATGCATGC CACCATGCAG CGGGCGCGCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG
68
69     GACTCCTGGA
70
71     (2) INFORMATION FOR SEQ ID NO:3:
72
73     (i) SEQUENCE CHARACTERISTICS:
74         (A) LENGTH: 24 base pairs
75         (B) TYPE: nucleic acid
76         (C) STRANDEDNESS: single
77         (D) TOPOLOGY: linear
78
79     (ii) MOLECULE TYPE: DNA (genomic)
80
81     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
82
83     ACATGCATGC CCCGCCGGTC ATCC
84
85     (2) INFORMATION FOR SEQ ID NO:4:
86
87     (i) SEQUENCE CHARACTERISTICS:
88         (A) LENGTH: 22 base pairs
89         (B) TYPE: nucleic acid
90         (C) STRANDEDNESS: single
91         (D) TOPOLOGY: linear
92
93     (ii) MOLECULE TYPE: DNA (genomic)
94
95     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
96
97     CGGAATTCCT CATGACCCCA AC
98
99     (2) INFORMATION FOR SEQ ID NO:5:
```

TEAM 7

PAGE: 1

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895

DATE: 04/11/96
TIME: 14:24:17

INPUT SET: S9820.raw

This Raw Listing contains the General
Information Section and up to the first 5 pages.

SEQUENCE LISTING

ENTERED

(1) General Information:

(i) APPLICANT: Alitalo, Kari
Joukov, Vladimir

(ii) TITLE OF INVENTION: Receptor Ligand

(iii) NUMBER OF SEQUENCES: 35

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
(B) STREET: 6300 Sears Tower, 233 South Wacker Drive
(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606-6402

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Gass, David A.
(B) REGISTRATION NUMBER: 38,153
(C) REFERENCE/DOCKET NUMBER: 28113/33072

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 312/474-6300
(B) TELEFAX: 312/474-0448
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

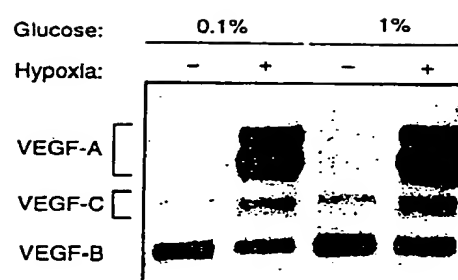


FIG. 18

FIG. 17

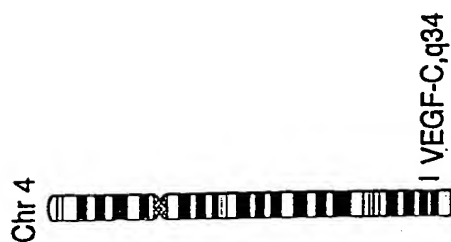


FIG. 15B

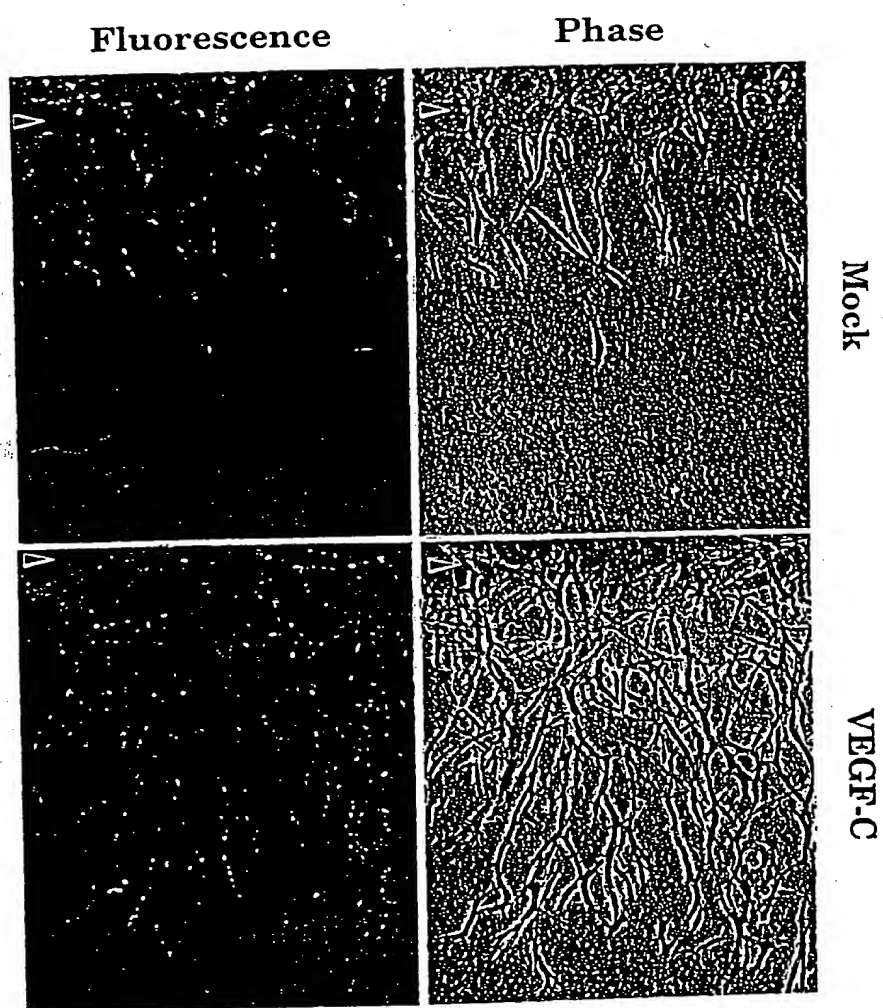


FIG. 15A

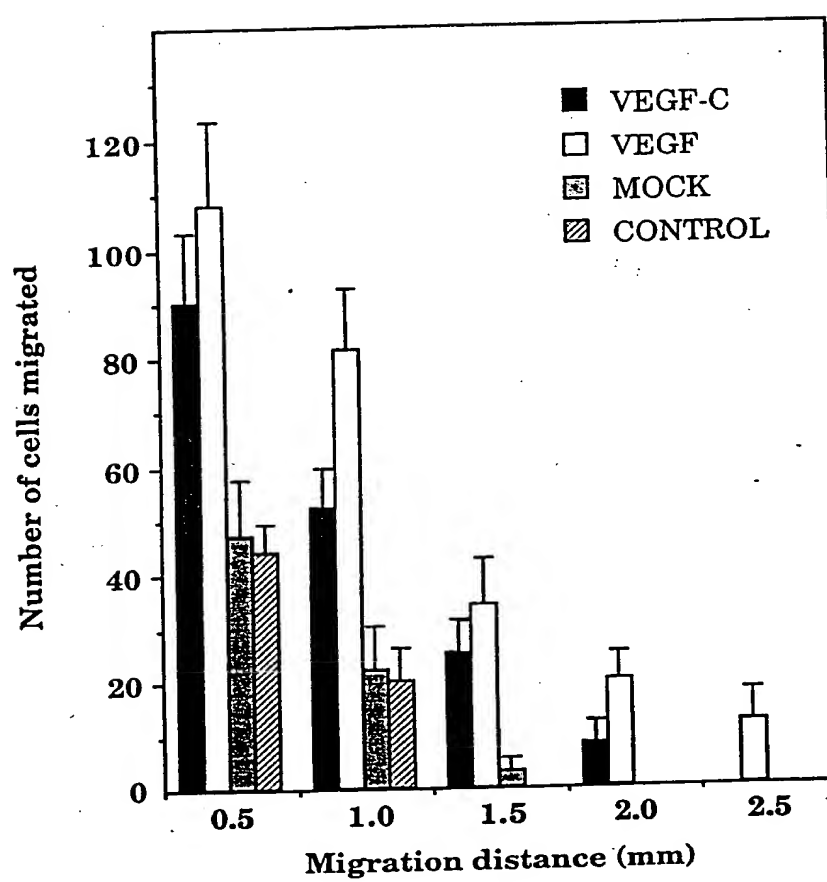


FIG. 14B

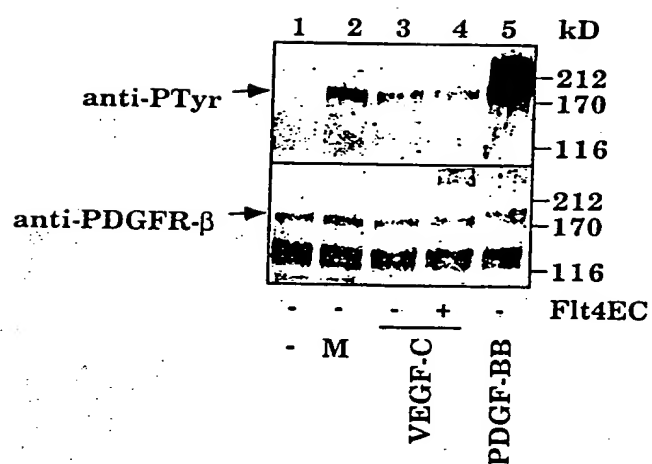


FIG. 14A

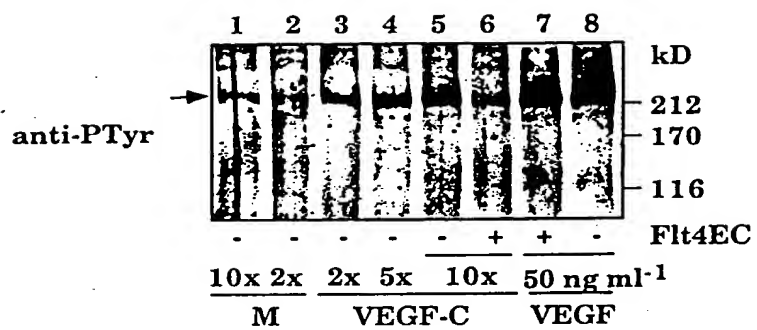
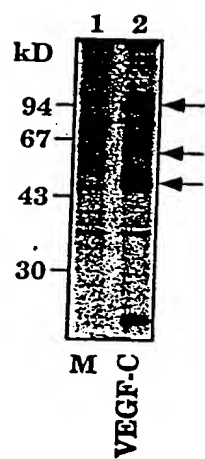


FIG. 13B



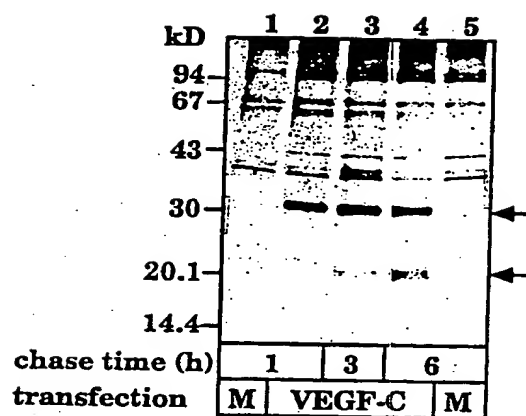


FIG. 13A

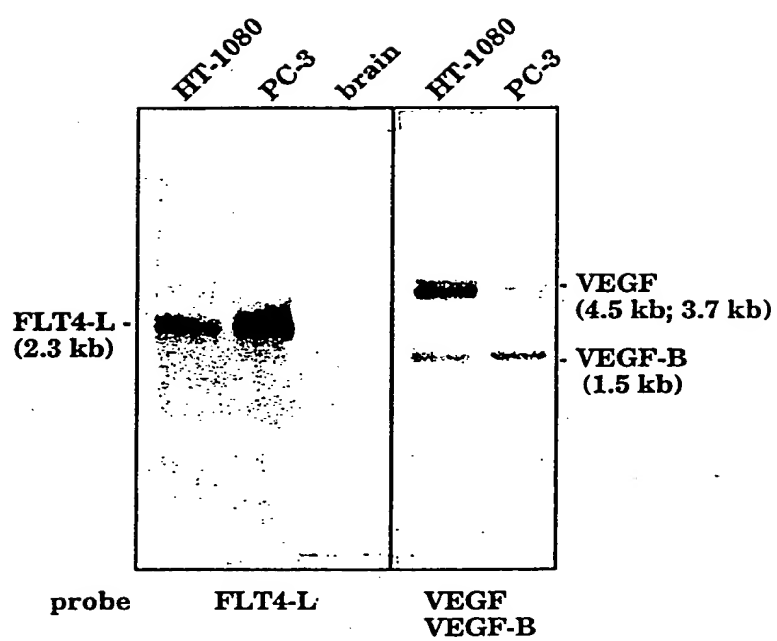


FIGURE 12

00 58589

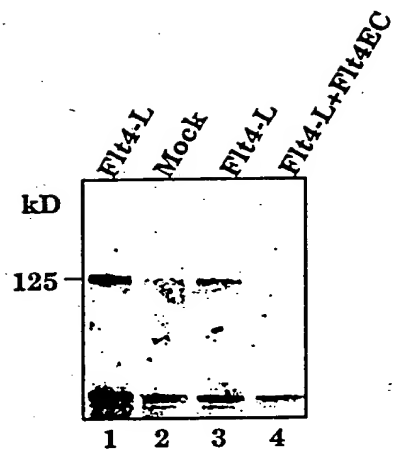


FIGURE 11

	251				300
PDGF-A
PDGF-B
PlGF-1
PlGF-2
VEGF121
VEGF165	CGPCSEERRKH	LFVQDPQTCK	CSCKNIDSRG	KARQLELNER	TCRCDKPRR.
VEGF189	CGPCSEERRKH	LFVQDPQTCK	CSCKNIDSRG	KARQLELNER	TCRCDKPRR.
VEGF206	CGPCSEERRKH	LFVQDPQTCK	CSCKNIDSRG	KARQLELNER	TCRCDKPRR.
Flt4-L	FHDICGPNKE	LDEETCQCVC	RAGLRPASCG	PHKELDRNSC	QCVCCKNLFP
	301				350
PDGF-A
PDGF-B
PlGF-1
PlGF-2
VEGF121
VEGF165
VEGF189
VEGF206
Flt4-L	SQCCANREFD	ENTCQCVCCK	TCPRNQPLNP	GKCAECTES	PQRCLLKCKK
	351				394
PDGF-A
PDGF-B
PlGF-1
PlGF-2
VEGF121
VEGF165
VEGF189
VEGF206
Flt4-L	FHHQTCSCYR	RPCTNRQKAC	EPGFSYSEEV	CRCVPSYWKR	PQMS

FIG. 10 B

	1		50
PDGF-A	.MRTWACLLL LGCGYLAHAL AEEAEIPREL IERLARSQIH SIRDOLQRLLE		
PDGF-B	MNRCAW.LFL SLCCYLRLVS AEGDPIPEEL YEMLSDSHSIR SFQDLQRLLE		
PlGF-1MPVM RLFPFC..FLQ LLAGLAL...		
PlGF-2MPVM RLFPFC..FLQ LLAGLAL...		
VEGF121M NFLLS..WVH WSLALLLYLH		
VEGF165M NFLLS..WVH WSLALLLYLH		
VEGF189M NFLLS..WVH WSLALLLYLH		
VEGF206M NFLLS..WVH WSLALLLYLH		
Flt4-LM TVLYPEYWKY YKQCLRKGGW		
	51		100
PDGF-A	IDSUGAEDAL ETSLRANGSH AINHVPKRP VPIRRKRSI.EEAIP		
PDGF-B	GDP.GEEDGA ELDLNMTRSH SGGELES... .LARGRRSLG SLTIAEPAMI		
PlGF-1	PAVPPQQW... .ALSAC NGSSEVEVVP FQE.VWGR...		
PlGF-2	PAVPPQQW... .ALSAC NGSSEVEVVP FQE.VWGR...		
VEGF121	HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...		
VEGF165	HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...		
VEGF189	HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...		
VEGF206	HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...		
Flt4-L	QHNREQANLN SRTEETIKFA AAHYNTEILK SIDNEWK...		
	101		150
PDGF-A	AVCKIRTVIY EIPRSQVDPT SANFLIWPPC VEVKRCGTGCC NTSSVKCQPS		
PDGF-B	AECKIRTEVF EISRRLLDRT NANFLVWPPC VEVQRCSGCC NNRNVQCRPT		
PlGF-1	SYCRALERLV DUVSEYPS.. EVEHMFSPSC VSLLRCTGCC GDENLHCVPV		
PlGF-2	SYCRALERLV DUVSEYPS.. EVEHMFSPSC VSLLRCTGCC GDENLHCVPV		
VEGF121	SYCHPIETLV DIFQEYPD.. EIEYIFKPSC VPLMRCGGCC NDEGLECVPT		
VEGF165	SYCHPIETLV DIFQEYPD.. EIEYIFKPSC VPLMRCGGCC NDEGLECVPT		
VEGF189	SYCHPIETLV DIFQEYPD.. EIEYIFKPSC VPLMRCGGCC NDEGLECVPT		
VEGF206	SYCHPIETLV DIFQEYPD.. EIEYIFKPSC VPLMRCGGCC NDEGLECVPT		
Flt4-L	TQCMPEVCI DVGKEFGV.. ATNTFFKPPC VSVYRCGGCC NSEGLQCMNT		
	151		200
PDGF-A	RVVHRSVKVA KVEYVRKKPK LKEVQVRLEE HLEACAT..SN		
PDGF-B	QVQLRPVQVR KIEIVRKPKI FKKATVTLED HLACKCETVA AARPVTRSPG		
PlGF-1	ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRCECRPLR EKMKPER...		
PlGF-2	ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRCECRPLR EKMKPERRR.		
VEGF121	EESNITMQIM RIKPH..QQG .HIGEMSFLQ HNKCECRPKK DRARQEKCD.		
VEGF165	EESNITMQIM RIKPH..QQG .HIGEMSFLQ HNKCECRPKK DRARQEN...		
VEGF189	EESNITMQIM RIKPH..QQG .HIGEMSFLQ HNKCECRPKK DRARQEKKS.		
VEGF206	EESNITMQIM RIKPH..QQG .HIGEMSFLQ HNKCECRPKK DRARQEKKS.		
Flt4-L	STSYLSKTLF EITVPLSQSP .KPVITISFAN HTSCRCMSKL DVYRQVHSII		
	201		250
PDGF-A	LNPDHREET DVR.....		
PDGF-B	GSQEORAKTP QTRVTIRTVR VRRPPKGGHR KFKHTHDKTA LKETLGA...		
PlGF-1CGDAVPR R.....		
PlGF-2PKGRGK RRREKQRPD CHLCGDAVPR R.....		
VEGF121KPRR.....		
VEGF165VRGKGK GQKRKRKKS R YKWSV.....		P
VEGF189VRGKGK GQKRKRKKS R YKWSV.....		P
VEGF206VRGKGK GQKRKRKKS R YKWSV.....		P
Flt4-L	RRSLPATLPQ CQAANKTCPT NYMWNHICR CLAQEDFMFS SDAGDDSTDG		

FIG. 10A

	251				300
PDGF-A
PDGF-B
PlGF-1
PlGF-2
VEGF121
VEGF165	CGPCSEERRKH	LFVQDPQTCK	CSCKNNTDSRC	KARQLELNER	TCRCDKPRR.
VEGF189	CGPCSEERRKH	LFVQDPQTCK	CSCKNNTDSRC	KARQLELNER	TCRCDKPRR.
VEGF206	CGPCSEERRKH	LFVQDPQTCK	CSCKNNTDSRC	KARQLELNER	TCRCDKPRR.
Flt4-L	FHDICGPNKE	LDEETCQVC	RAGLRPASC	PHKELDNRSC	QCVCKNKLFP
	301				350
PDGF-A
PDGF-B
PlGF-1
PlGF-2
VEGF121
VEGF165
VEGF189
VEGF206
Flt4-L	SQCGANREFD	ENTCQCVCCKR	TCPRNQPLNP	GKCACECTES	PQKCLLLGKK
	351				394
PDGF-A
PDGF-B
PlGF-1
PlGF-2
VEGF121
VEGF165
VEGF189
VEGF206
Flt4-L	FHHQTCSCYR	RPCTNRQKAC	EPGFSYSEEV	CRCVPSYWKR	PQMS

FIG. 10

1
 PDGF-A .MRTWACLLL LGCCYLALAL AEEAEIPREL IERLARSQIH SIRDLOQLLE
 PDGF-B MNRCWA.LFL SLCCYLRLVS AEGDPIPEEL YEMLSDHSIR SFDDLQRLH
 P1GF-1MPVM RLFPCL..FLQ LLAGLAL...
 P1GF-2MPVM RLFPCL..FLQ LLAGLAL...
 VEGF121M NFFLS..WVH WSLALLLYLH
 VEGF165M NFFLS..WVH WSLALLLYLH
 VEGF189M NFFLS..WVH WSLALLLYLH
 VEGF206M NFFLS..WVH WSLALLLYLH
 Flt4-LM TVLYPEYWKM YKQCLRKGW

51
 PDGF-A IDSVGAEDAL ETSLEHAGSH AINHVPEKRP VP1RRKRSI.EEAIP
 PDGF-B GDP.GEEDGA ELDLNMTRSH SGGELES... .LARGRRSLG SLTIAEPAMI
 P1GF-1 PAVPPQW... .ALSAG NGSSEVEVVP FQE.VWGR...
 P1GF-2 PAVPPQW... .ALSAG NGSSEVEVVP FQE.VWGR...
 VEGF121 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...
 VEGF165 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...
 VEGF189 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...
 VEGF206 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...
 Flt4-L QHNREQANLN SRTEETIKFA AAHYNTEILK SIDNEWK...

101
 PDGF-A AVCKTRTVIY EIPRSQVDPT SANFLIWPPC VEVRKCTGCC NTSSVKCQPS
 PDGF-B AECKTRTEVF EISRLIDRT NANFLVWPPC VEVRQCSGCC NNRNVQCRPT
 P1GF-1 SYCRALERLV DVVSEYPS... EVEHMFSPSC VSLLRCTGCC GDENLHCVPV
 P1GF-2 SYCRALERLV DVVSEYPS... EVEHMFSPSC VSLLRCTGCC GDENLHCVPV
 VEGF121 SYCHPIETLV DIFQEYPD... EIEYIFKPCSC VPLMRGCGCC NDEGLECVPT
 VEGF165 SYCHPIETLV DIFQEYPD... EIEYIFKPCSC VPLMRGCGCC NDEGLECVPT
 VEGF189 SYCHPIETLV DIFQEYPD... EIEYIFKPCSC VPLMRGCGCC NDEGLECVPT
 VEGF206 SYCHPIETLV DIFQEYPD... EIEYIFKPCSC VPLMRGCGCC NDEGLECVPT
 Flt4-L TQCMPREVCI DVGKEFGV... ATNTFFKPPC VSVYRCGCC NSEGLQCMNT

151
 PDGF-A RVHHRSVKVA KVEYVRKKPK LKEVQVRLEE HLEACAT... ..SN
 PDGF-B QVQLRPVQVR KIEIVRKKPI FKATVTLED HLACKCETVA AARPVTRSPG
 P1GF-1 ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRCECRPLR EKMKPER...
 P1GF-2 ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRCECRPLR EKMKPER...
 VEGF121 EESNITMQIM RIKPH..QQQ .HIGEMSFLQ HNKCECRPKK DRARQEKCD.
 VEGF165 EESNITMQIM RIKPH..QQQ .HIGEMSFLQ HNKCECRPKK DRARQEN...
 VEGF189 EESNITMQIM RIKPH..QQQ .HIGEMSFLQ HNKCECRPKK DRARQEKKS.
 VEGF206 EESNITMQIM RIKPH..QQQ .HIGEMSFLQ HNKCECRPKK DRARQEKKS.
 Flt4-L STSYLSKTLF EITVPLSQGP .KPVITISFAN HTSCRCMSKL DVYRQVHSII

201
 PDGF-A LNPDHREEET DVR.....
 PDGF-B GSQEORAKTP QTRVTIRTVR VRRPPKGKHR KFKHTHDKTA LKETLGA...
 P1GF-1CGDAVPR R.....
 P1GF-2PKGRGK RRREKQRPTD CHLCGDAVPR R.....
 VEGF121KPRR.....
 VEGF165
 VEGF189VRGKGK GQKRKRKKS R YKSWSV.....
 VEGF206VRGKGK GQKRKRKKS R YKSWSVYVGA RCC.....L MPWSLPGPHD
 Flt4-L RRSPLPATLPQ CQAANKTCPT NYMWNHICR CLAQEDFMFS SDAGDDSTDG

FIG. 10

MetThrValLeuTyrProGluTyr
GAGCAGTTACCGTCTGCTGCCACTGTAGATGAACATCATGACTGTACTCTTACCCAGAAATAT
10 30 50
TrpLysMetTyrLysCysGlnLeuArgLysGlyGlyTrpGlnHisAsnArgGluGlnAla
TGC AAAATGTACAAAGTGTGCTAGCTAAGGAAAGAGGAGGCTGGCAACATAACACAGAACAGGCC
70 90 110
AsnLeuAsnSerArgThrGluGluTrpIleLysPheAlaAlaHisTyrAsnThrGlu
AACCTCAACTCAAGCAGACAGACAGACTATAAAATTTGCTGCCACCATTTATAATACAGAC
130 150 170
IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys
ATCTTGAAAAGTATTGATAATGAGTGGAGAAAGACTCAATGCATGCCACGGGAGGTGTGT
190 210 230
IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProProCysVal
ATAGATCTCGCCAGGAGTTTGGAGTCCGCCAACACCTTCTTTAAACCTCCATGTGTG
250 270 290
SerValTyrArgCysGlyGlyCysCysAsnSerGluGlyLeuGlnCysMetAsnThrSer
TCCGCTACACAGTCTCCCGCTTCTGCAATAGTACGGGCTGCAGTGCATGAACACCACG
310 330 350
ThrSerTyrLeuSerLysCysThrLeuPheGluIleThrValProLeuSerGlnGlyProLys
ACGAGCTACCTCAGCAACAGCTTATTGTAAATTTACAGTGCCTCTCTCTACAGGCCCCAAA
370 390 410
ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal
CCAGTAACAATCAGTTTGTCCCAATCACACTTCTTCCGATGCATGCTAAACTCGGATGTT
430 450 470
TyrArgGlnValHisSerIleIleArgArgSerLeuProAlaThrLeuProGlnCysGln
TACAGACAAGTTTCATTCATTATTAGACGTTTCCCTGCCAGCAACACTACCACAGTGTCTAG
490 510 530
AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu
GCAGCGCAAGACCTGCCCCACCAATTACATGTGGAATTAACATCTGCAGATGCTGTG
550 570 590
AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspAspSerThrAspGlyPheHis
GCTCAGCAAGATTTTATGTTTTCCTCGCATCTCGGAGATGACTCAACAGATCGATTCCAT
610 630 650
AspIleCysGlyProAsnLysGluLeuAspGluGluThrCysGlnCysValCysArgAla
CACATCTGTGGACCAAAACAGGAGCTGGAATGAAGACAGACTGTCACTGTCTCTCGAGACGG
670 690 710
GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys
GGGCTTCGGCTGCCAGCTGTGGACCCCAACAAAGACTAGACAGAACTCATGCCAGTGT
730 750 770
ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAla
GCTGTGTA AAAACAACTCTCCCAAGCATGTGGGGCCAAACCGCAAGTTTGATCAAAC
790 810 830
ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys
ACATGCCAGTGTGTATGTAAAGAACCTGCCCAAGAAATCAACCCCTAAATCTCGAAAAA
850 870 890
CysAlaCysGluCysThrGluSerProGlnLysCysLeuLeuLysGlyLysLysPheHis
TGTGCTGTGAATGTACAGAAAGTCCACAGAAATGCTTGTATAAAGGAAAGAGTTCCAC
910 930 950
HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro
CACC AAACATCCAGCTGTTACAGACGGCCATGACGAACCGCCAGAAGCTTGTGAACCA
970 990 1010
GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln
GGATTTTCATATAGTCAAGAAGCTGTGCTGTGCTCCTTCATATTGGAAGACCCACAA
1030 1050 1070
MetSerEnd
ATGAGCTAAGATTGTACTGTTTTCAGTTTCATCGATTTCCTATTATCGAAAACCTGTGTTG
1090 1110 1130

FIG. 9B

FIG. 9A

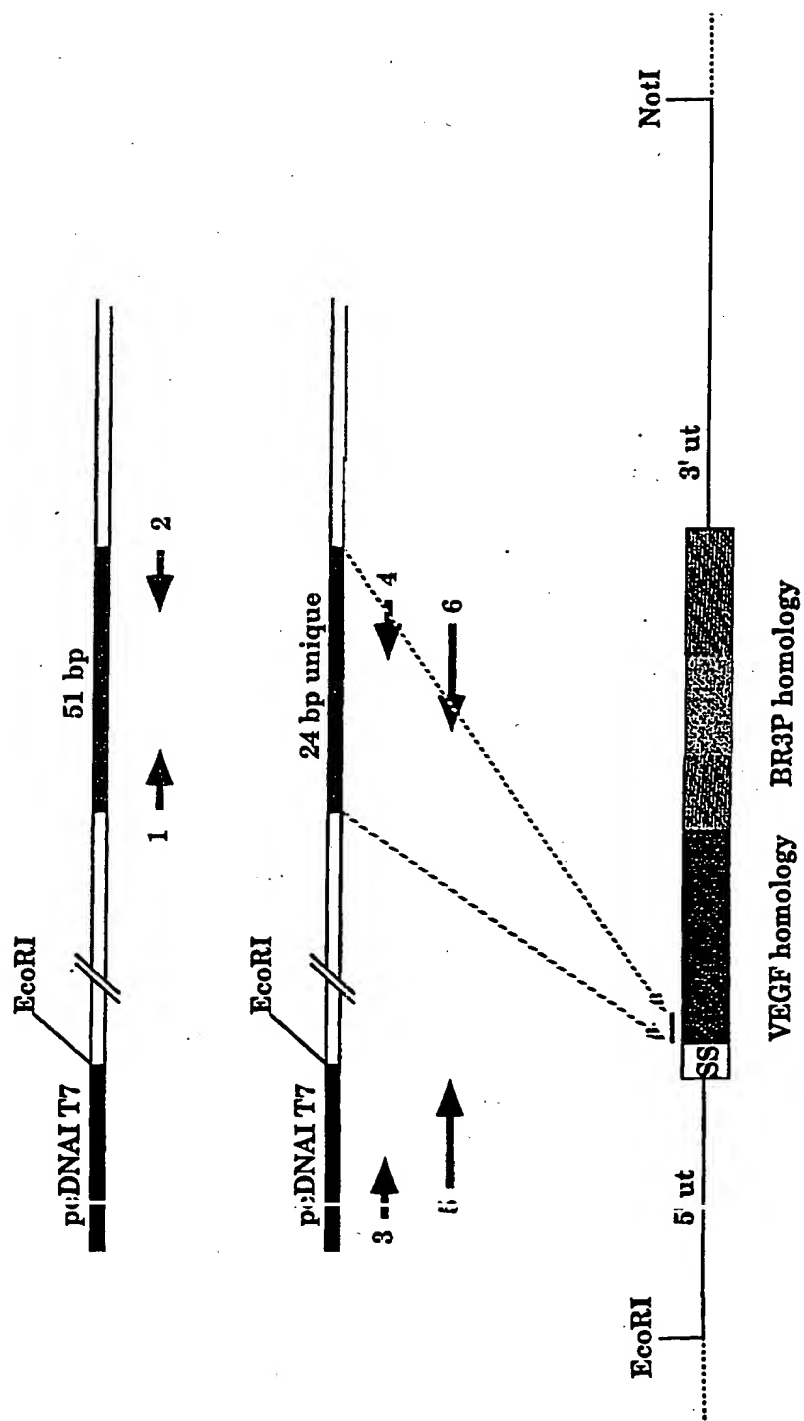
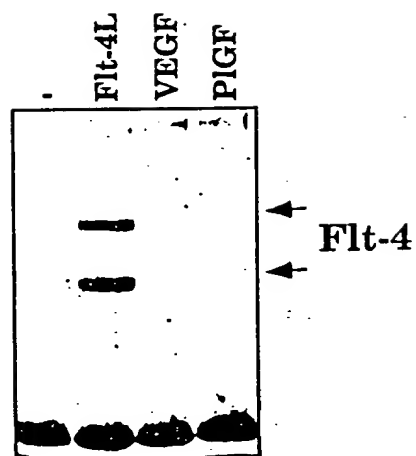
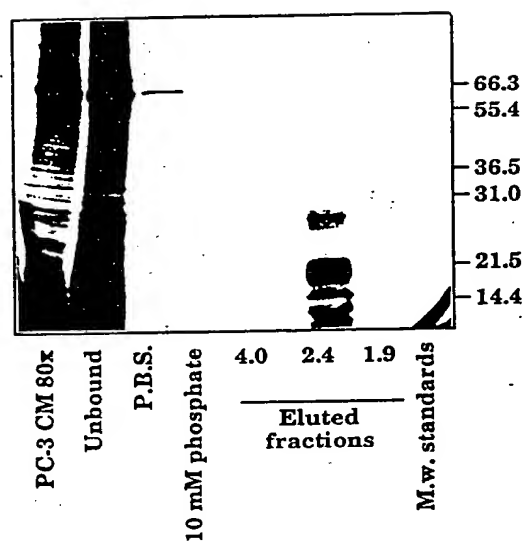


FIGURE 8



08 585895

FIGURE 7



08 585895

FIGURE 7

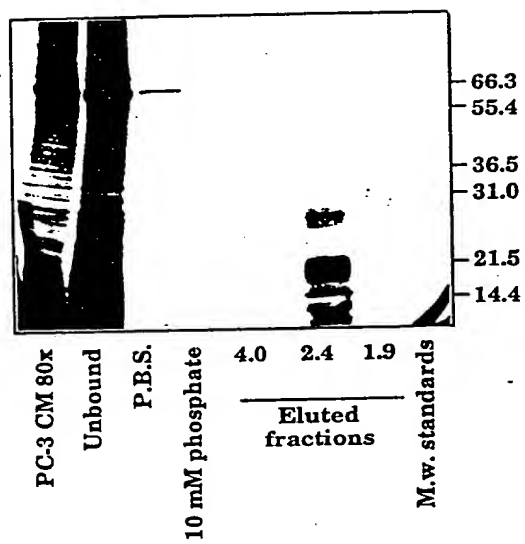
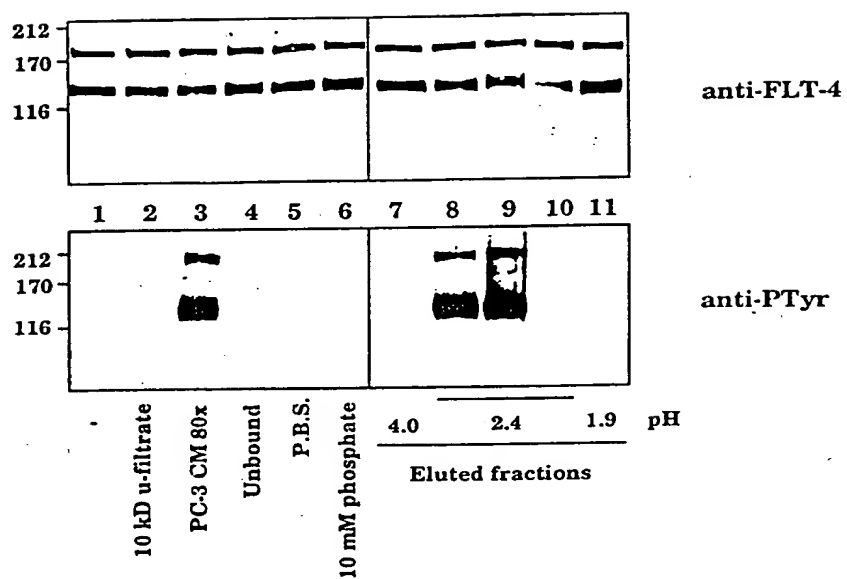


FIGURE 6



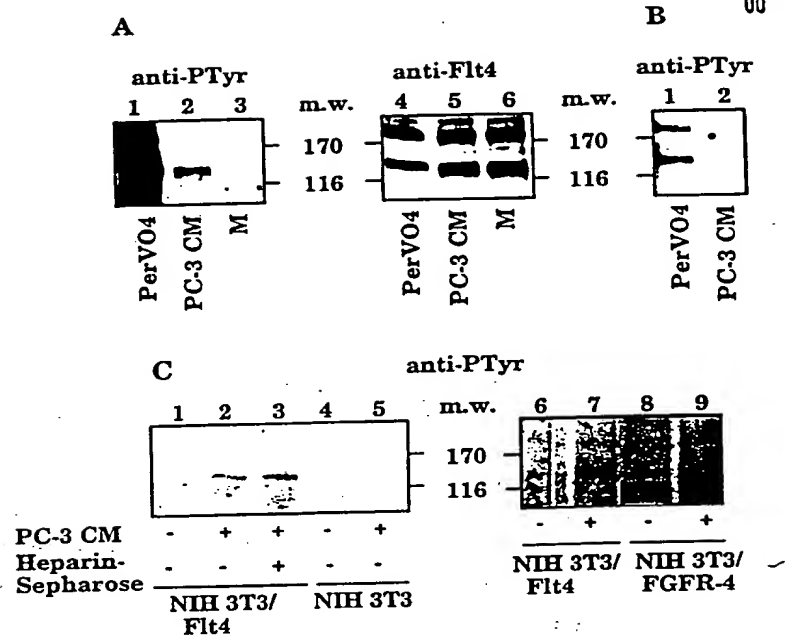


FIGURE 5

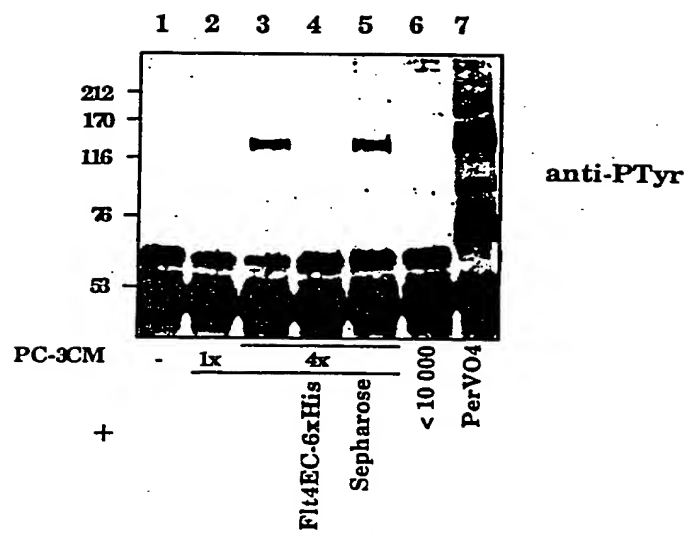
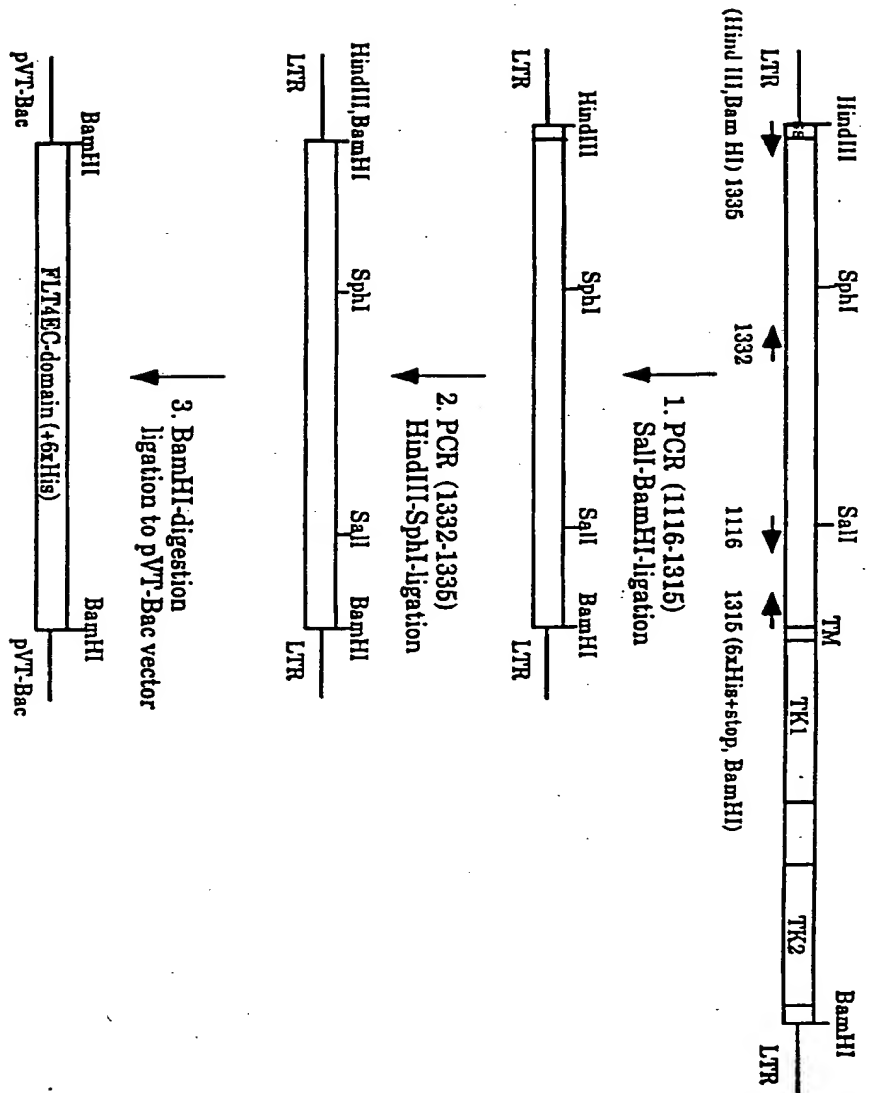


FIGURE 4

Figure 3



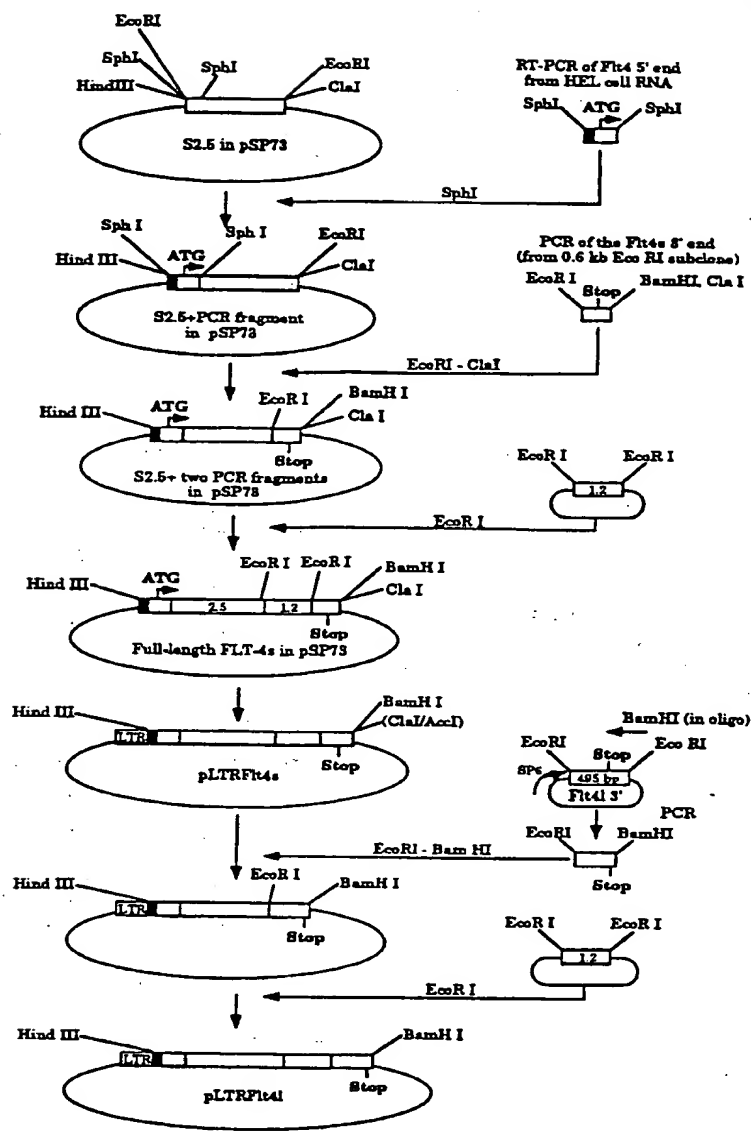


FIGURE 2

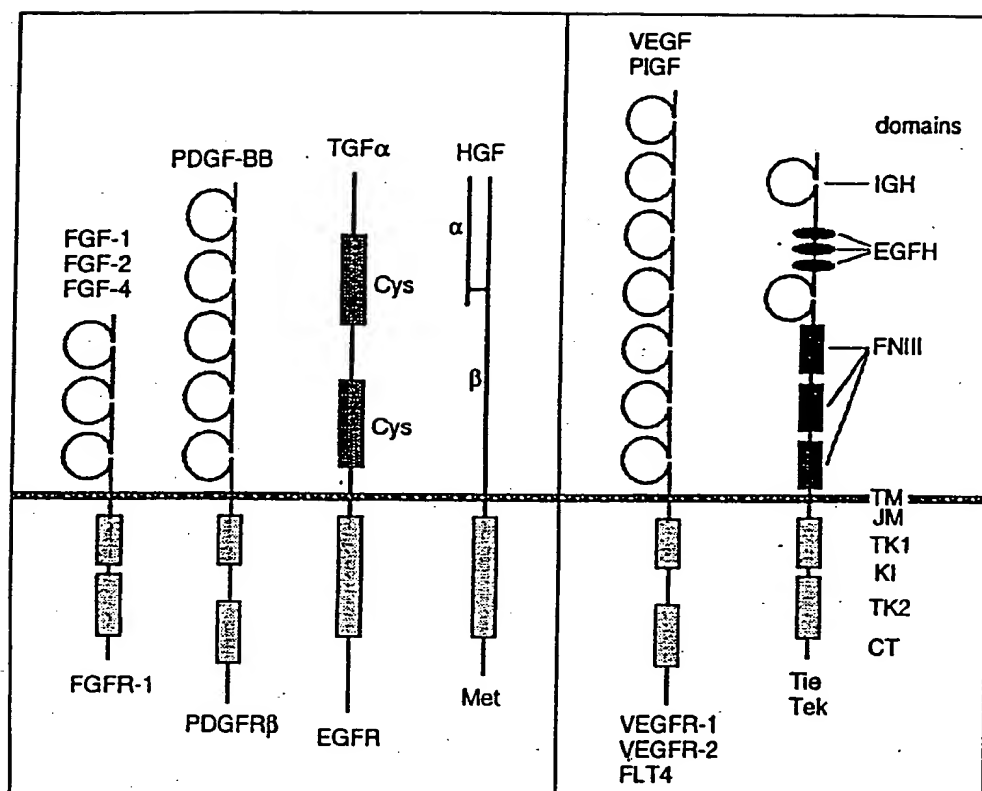


FIGURE 1

-51-

12. The fragment according to claim 8 comprising approximately amino acids 1-180 shown in SEQ ID NO: 33.

Sub B51

13. A purified and isolated nucleic acid encoding the fragment of claim 12.

14. An antibody which is specifically reactive with the Flt4 ligand.

15. An antibody of claim 14 which is a monoclonal antibody.

16. A pharmaceutical composition comprising a polypeptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

add B6

add CP

add D12

CLAIMS

- Sub B1
1. A purified and isolated polypeptide which specifically binds to the Flt4 receptor tyrosine kinase.
 2. A purified and isolated polypeptide having the amino acid sequence shown in SEQ ID NO: 33.
 3. A purified and isolated nucleic acid encoding the peptide according to claim 2.
 4. The nucleic acid according to claim 3 having the sequence shown in SEQ ID NO: 32.
 5. A vector comprising the nucleic acid according to claim 4.
 6. The vector according to claim 5, wherein said vector is plasmid pFLT4-L, deposited as ATCC accession No. 97231.
 7. A host cell comprising the vector according to claim 6.
 8. A fragment of the purified and isolated polypeptide according to claim 2, said fragment being capable of specifically binding to an Flt4 receptor tyrosine kinase.
 9. The fragment according to claim 8 having an apparent molecular weight of 23 kD under reducing conditions.
 10. The fragment according to claim 8 comprising approximately amino acids 1-120 of SEQ ID NO: 33.
 11. A purified and isolated nucleic acid encoding the fragment of claim 10.
- Sub B3
- Sub B4 CS
- Sub C6
- Sub B5

- 49 -

Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu
115 120 125
Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr
130 135 140
Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp
145 150 155 160
Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
165 170 175
Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys
180 185 190
Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu
195 200 205
Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro
210 215 220
Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys
225 230 235 240
Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys
245 250 255
Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly
260 265 270
Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr
275 280 285
Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val
290 295 300
Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser
305 310 315

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TGAGTGATTGTAGCTGCTGTG

22

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TATTGCAGCCACCCCGGCTTT

22

CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC 774
 Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys
 200 205 210

CAG TGT GTC TGT AAA AAC AAA CTC TTC CCC AGC CAA TGT GGG GCC AAC 822
 Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn
 215 220 225 230

CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC 870
 Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys
 235 240 245

CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA 918
 Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr
 250 255 260

GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA 966
 Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln
 265 270 275

ACA TGC AGC TGT TAC AGA CGG CCA TGT ACG AAC CGC CAG AAG GCT TGT 1014
 Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys
 280 285 290

GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA 1062
 Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser
 295 300 305 310

TAT TGG AAA AGA CCA CAA ATG AGC TAA GATTGTACTG TTTCCAGTT 1109
 Tyr Trp Lys Arg Pro Gln Met Ser 315

CATCGATTTT CTATTATGGA AAACGTGTT G 1140

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 350 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu
 -32 -30 -25 -20

Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser
 -15 -10 -5

Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu
 1 5 10 15

Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro
 20 25 30

Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn
 35 40 45

Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys
 50 55 60

Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu
 65 70 75 80

Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys
 85 90 95

Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser
 100 105 110

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 37..1089

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 133..1089

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAAC	ATG ACT GTA CTC TAC CCA	54
	Met Thr Val Leu Tyr Pro	
	-32 -30	
GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA		102
Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln		
-25 -20	-15	
CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA		150
His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile		
-10 -5	1	5
AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT		198
Lys Phe Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp		
10 15	20	
AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT		246
Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp		
25 30	35	
GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA		294
Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro		
40 45	50	
TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG		342
Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu		
55 60	65	70
CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA		390
Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu		
75 80	85	
ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT		438
Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe		
90 95	100	
GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA		486
Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg		
105 110	115	
CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG		534
Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln		
120 125	130	
TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT		582
Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn		
135 140	145	150
CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT		630
His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp		
155 160	165	
GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC		678
Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn		
170 175	180	
AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT		726
Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu		
185 190	195	

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCTAGCATT AGGTGACAC

19

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AAGAGACTAT AAAATTGCT GCAGC

25

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCTCTAGAT GCATGCTCGA

20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1140 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
GTTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Phe Ala Ala Ala His Tyr Asn
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 219 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCACTATAGG GAGACCCAAG CTTGGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT
GTGGTGGAAT TCGACGAACT CATGACTGTA CTCTACCCAG AATATTGGAA AATGTACAAG
TGTGAGCTAA GGCAAGGAGG CTGGCAACAT AACAGAGAAC AGGCCAACCT CAACTCAAGG
ACAGAAGAGA CTATAAAATT CGCTGCAGCA CACTACAAC

60

120

180

219

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACAGGAGAGC GGGCAAGC

18

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid

Thr Glu Ile Leu Lys
1 5

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTCGCTGCA GCACACTACA AC

22

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCNGTGTGT AGTGTGCTG

19

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Ala His Tyr Asn Thr Glu
1 5

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TAATACGACT CACTATAGGG

20

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCAGARGARA CNATHAA

17

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Glu Thr Ile Lys
1 5

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAYTTNARD ATYTCNGT

18

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
1 5 10 15
Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
20 25 30
His Arg Gln Glu Ser Gly Phe Arg
35 40

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGAGTCGA CTGGCGGAC T

21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTATCCTC

60

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC

34

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTTGCCTGTG ATGTGCACCA

20

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
 ACATGCATGC CCCGCCGGTC ATCC 24
- (2) INFORMATION FOR SEQ ID NO:4:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
 CGGAATTCCC CATGACCCCA AC 22
- (2) INFORMATION FOR SEQ ID NO:5:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
 CCATCGATGG ATCTACCTG AAGCCGCTT CTT 33
- (2) INFORMATION FOR SEQ ID NO:6:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
 ATTTAGGTGA CACTATA 17
- (2) INFORMATION FOR SEQ ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
 CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT 34
- (2) INFORMATION FOR SEQ ID NO:8:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 amino acids



- 40 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Alitalo, Kari
Joukov, Vladimir
- (ii) TITLE OF INVENTION: Receptor Ligand
- (iii) NUMBER OF SEQUENCES: 35
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
(B) STREET: 6300 Sears Tower, 233 South Wacker Drive
(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Gass, David A.
(B) REGISTRATION NUMBER: 38,153
(C) REFERENCE/DOCKET NUMBER: 28113/33072
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312/474-6300
(B) TELEFAX: 312/474-0448
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTCCTCGCT GTCCTGTCT

20

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG
GACTCCTGGA

60

70

(2) INFORMATION FOR SEQ ID NO:3:

isolated (as in the other examples), and 8 micrograms of the RNA was electrophoresed and blot-hybridized with a mixture of the VEGF, VEGF-B and VEGF-C probes (see Fig. 12). The results show that hypoxia strongly induces VEGF-A mRNA expression (compare lanes - and +), both in low and high glucose, but has no significant effect on the VEGF-B mRNA levels. The VEGF-C mRNA isolated from hypoxic cells runs slightly faster in gel electrophoresis and an extra band of faster mobility can be seen below the upper mRNA band. This observation suggests that hypoxia affects VEGF-C RNA processing. One explanation for this observation is that VEGF-C mRNA splicing is altered, affecting the VEGF-C open reading frame and resulting in an alternative VEGF-C protein being produced by hypoxic cells. Such alternative forms of VEGF-C and VEGF-C-encoding polynucleotides are contemplated as an aspect of the invention.

Deposit of Biological Materials: Plasmid FLT4-L has been deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville MD ²⁰⁸⁵² 20952 (USA), pursuant to the provisions of the Budapest Treaty, and has been assigned a deposit date of 24 July 1995 and ATCC accession number 97231.

While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those in the art. Accordingly, only such limitations as appear in the appended claims should be placed on the invention.

In order to determine the chromosomal localization of the human VEGF-C gene, DNAs from human rodent somatic cell hybrids containing defined sets of human chromosomes were ^{analysed} by Southern blotting and hybridization with the VEGF-C cDNA probe. Among 24 DNA samples on the hybrid panel, representing different human chromosomes, human-specific signals were observed only in hybrids which contained human chromosome 4. The results were confirmed by PCR of somatic cell hybrid DNA using VEGF-C specific primers, where amplified bands were obtained only from DNAs containing human chromosome 4.

A genomic P1 plasmid for VEGF-C was isolated using specific primers and PCR and verified by Southern blotting and hybridization using a VEGF-C specific cDNA probe. The chromosomal localization of VEGF-C was further studied using metaphase FISH. Using the P1 probe for VEGF-C in FISH a specific hybridization to the 4q34 chromosomal band was detected in 40 out of 44 metaphases (Fig. 17). Double-fluorochrome hybridization using a cosmid probe specific for the aspartylglucosaminidase (AGA) gene showed that VEGF-C is located just proximal to the AGA gene previously mapped to the 4q34-35 chromosomal band.

Biotin labelled VEGF-C P1 and digoxigenin labeled AGA cosmid probes were hybridized simultaneously to metaphase chromosomes. This experiment demonstrated that the AGA gene is more telomerically located than the VEGF-C gene. The foregoing example demonstrates the utility of polynucleotides of the invention as chromosomal markers.

EXAMPLE 18

Effect of glucose concentration and hypoxia on VEGF, VEGF-B and VEGF-C mRNA levels in C6 glioblastoma cells

Confluent cultures of C6 cells (ATCC CCL 107) were grown on 10 cm diameter tissue culture plates containing 2.5 ml of DMEM and 5% fetal calf serum plus antibiotics. The cultures were exposed for 16 hours to normoxia in a normal cell culture incubator containing 5% CO₂ (Fig. 18: lanes marked -) or hypoxia (Fig. 18: lanes marked +) by closing the culture plates in an airtight glass chamber and burning a piece of wood inside until the flame was extinguished due to lack of oxygen. Polyadenylated RNA was

domain was used as a probe in Southern blotting and hybridization analysis of the somatic cell hybrid DNAs as instructed by the supplier (Bios Laboratories).

The cell lines for fluorescence *in situ* hybridization (FISH) were obtained from the American Type Culture Collection (Rockville, MD).

5 Purified DNA from P1 clones 7660 and 7661 (VEGF-C) (Genome Systems, Inc., St. Louis, MO) were confirmed positive by Southern blotting of Eco RI-digested DNA followed by hybridization with the VEGF-C cDNA. The P1 clones were then labelled by nick translation either with biotin-11-dUTP, biotin-14-ATP (Sigma Chemical Co., St. Louis, MO) or digoxigenin 11-dUTP
10 (Boehringer Mannheim GmbH, Mannheim, Germany) according to standard protocols. PHA-stimulated peripheral blood lymphocyte cultures were treated with 5-bromodeoxyuridine (BrdU) at an early replicating phase to induce G-banding. See Takahashi *et al.*, *Human Genet.*, 86:14-16 (1995); Lemieux *et al.*, *Cytogenet. Cell Genet.*, 59:311-12 (1992). The FISH procedure was
15 carried out in 50% formamide, 10% dextran sulphate in 2x SSC using well-known procedures. See, e.g., Rytkönnen *et al.*, *Cytogenet. Cell Genet.*, 68:61-63 (1995); Lichter *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:9664-68 (1988). Repetitive sequences were suppressed with 50-fold excess of Cot-1
C DNA (BRL, ^{Martinsburg} Gaithersburg, MD) compared with the labeled probe. Specific
20 hybridization signals were detected by incubating the hybridized slides in labelled antidigoxigenin antibodies, followed by counterstaining with 0.1mmol/L 4,6-diamino-2-phenylindole. Probe detection for two-color experiments was accomplished by incubating the slides in fluorescein isothiocyanate (FITC)-anti-digoxigenin antibodies (Sigma Chemical Co.) and
25 Texas red-avidin (Vector Laboratories, Burlingame, CA) or rhodamine-anti-digoxigenin and FITC-avidin.

Multi-color digital image analysis was used for acquisition, display and quantification of hybridization signals of metaphase chromosomes. The system contains a PXL camera (Photometrics Inc., Tucson, AZ) attached
30 to a PowerMac 7100/Av workstation. IPLab software controls the camera operation, image acquisition and Ludl Filter wheel. At least 50 nuclei were scored. Overlapping nuclei and clusters of cells were ignored. A slide containing normal lymphocyte metaphase spreads and interphase nuclei was included in each experiment to control for the efficiency and specificity of the
35 hybridization.

example of typical phase contrast and fluorescent microscopic fields of cultures stimulated with medium from mock-transfected or VEGF-C transfected cells is shown in Fig. 15B. Daily addition of 1 ng of FGF2 into the wells resulted in the migration of approximately twice the number of cells when compared to the stimulation by CM from VEGF-transfected cells.

EXAMPLE 16

VEGF-C Is Expressed In Multiple Tissues

Northern blots containing 2 micrograms of isolated poly(A)⁺ RNA from multiple human tissues (blot from Clontech) were probed with radioactively labelled insert of the 2.0 kb VEGF-C cDNA clone. Northern blotting and hybridization analysis showed that the 2.4 kb RNA and smaller amounts of a 2.0 kb mRNA are expressed in multiple human tissues, most prominently in the heart, placenta, muscle, ovary and small intestine (Fig. 16A). Very little VEGF-C RNA was seen in the brain, liver or thymus and peripheral blood leukocytes (pbl) appeared negative. A similar analysis of RNA from human fetal tissues (Fig. 16B) shows that VEGF-C is highly expressed in the kidney and lung and to a lesser degree in the liver, while essentially no expression is detected in the brain. Interestingly, VEGF expression correlates with VEGF-C expression in these tissues, whereas VEGF-B is ^{highly} expressed in all tissues ^{analysed}.

EXAMPLE 17

The VEGF-C Gene Localizes To Chromosome 4q34

A DNA panel of 24 interspecies somatic cell hybrids, which had retained one or two human chromosomes, was used for the chromosomal localization of the VEGF-C gene (Bios Laboratories, Inc., New Haven, CT). Primers were designed to amplify an about 250 bp fragment of the VEGF-C gene from somatic cell hybrid DNA. The primers and conditions for polymerase chain reaction (PCR) were 5'-TGAGTGATTGTAGCTGCTGTG-3' (forward) [SEQ ID NO:34] and 5'-TATTGCAGCAACCCCCACATCT-3' (reverse) [SEQ ID NO:35] for VEGF-C (94°C, 60s/62°C, 45s/72°C, 60s). The PCR products were evaluated by electrophoresis in 1% agarose gels and visualized by ethidium bromide staining in ultraviolet light. [α -³²P]-dCTP-labelled cDNA inserts of a plasmid representing the complete VEGF-C coding

For the migration assays, the cells were allowed to attach inside a plastic ring (1 cm diameter) placed on top of the first collagen layer. After 30 min., the ring was removed and unattached cells were rinsed away. A second layer of collagen and a layer of growth medium (5% newborn calf serum (NCS)), solidified by 0.75% low melting point agar (FMC BioProducts, Rockland, ME), were added. A well (3 mm diameter) was punched through all the layers on both sides of the cell spot at a distance of 4 mm, and the sample or control media were pipetted daily into the wells. Photomicrographs of the cells migrating out from the spot edge were taken after six days through an Olympus CK 2 inverted microscope equipped with phase-contrast optics. The migrating cells were counted after nuclear staining with the fluorescent dye bisbenzimidazole (1 mg/ml, Hoechst 33258, Sigma).

Fig. 15A depicts a comparison of the number of cells migrating at different distances from the original area of attachment towards wells containing media conditioned by the non-transfected (control) or transfected (mock; VEGF-C; VEGF) cells, 6 days after addition of the media. The number of cells migrating out from the original ring of attachment was counted in five adjacent 0.5 mm x 0.5 mm squares using a microscope ocular lens grid and 10x magnification. Cells migrating further than 0.5 mm were counted in a similar way by moving the grid in 0.5 mm steps. The experiments were carried out twice with similar results, and medium values from the one of the experiments are presented with standard error bars. The photographs in Fig. 15B depict phase-contrast microscopy and fluorescent microscopy of the nuclear staining of BCE cells migrating towards the wells containing media conditioned by the mock-transfected cells or by VEGF-C - transfected cells. The areas shown is approximately 1mm x 1.5mm, and arrows indicate the borders of the original ring of attachment.

After 6 days of treatment, the cultures were stained and cells at different distances outside of the original ring of attachment were counted using fluorescent nuclear staining and detection with a fluorescence microscope equipped with a grid. A comparison of the numbers of migrating cells in successive 0.5 mm x 0.5 mm areas is shown in Fig 15A. As can be seen from the columns, VEGF-C-containing CM stimulated cell migration more than medium conditioned by the non-transfected or mock-transfected cells but less than medium from cells transfected with a VEGF expression vector. An

nonradioactive aminoterminal sequence analysis is isolated. The determination of the NH₂-terminal sequence of the carboxyl terminal fragment allows for identification of the proteolytic processing site. This is confirmed by site-directed mutagenesis of the amino acid residues adjacent to the cleavage site, which would prevent the cleavage.

On the other hand, the Flt4 ligand is characterized by progressive 3' deletions in the 3' coding sequences of the Flt4 ligand precursor clone, resulting in carboxy-terminal truncations of its protein product. The activities of such truncated forms are ^{assayed} assayed by, for example, studying Flt4 autophosphorylation induced by the truncated proteins when applied to cultures of cells, such as NIH3T3^{3T3} cells expressing LTRFlt4. By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin *et al.*, *Growth Factors* 8:245-252 (1993)) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues of the secreted VEGF-C protein lacking the signal sequence, and apparently within the first approximately 120 amino acid residues.

On the other hand, the difference between the molecular weights of the purified ligand and the open reading frame of the Flt4 precursor clone may be due to the fact that the soluble ligand was produced from an alternatively spliced mRNA which would also be present in the PC-3 cells, from which the isolated ligand was derived. To isolate such alternative cDNA clones one uses cDNA fragments of the deposited clone and PCR primers made according to the sequence provided as well as techniques standard in the art to isolate or amplify alternative cDNAs from the PC-3 cell cDNA library. One may also amplify using reverse transcription (RT)-PCR directly from the PC-3 mRNA using the primers provided in the sequence of the Flt4-L clone. Alternative cDNA sequences are determined from the resulting cDNA clones. One can also isolate genomic clones corresponding to the Flt4-L transcript from a human genomic DNA library using methods standard in the art and to sequence such clones or their subcloned fragments to reveal the corresponding exons. Alternative exons can then be identified by a number of methods standard in the art, such as heteroduplex analysis of cDNA and genomic DNA, which are subsequently be characterized.

Parklawn Drive, Rockville, MD 20852 as accession number 97231.

However, the predicted molecular weight of the mature protein product deduced from this reading-frame is 35881 and the Flt4 ligand from PC-3 cell cultures had an approximate molecular weight of 23 kD under reducing conditions. It is thus possible that the Flt4-L mRNA may be first translated into a precursor, from which the mature ligand is derived by proteolytic cleavage. The difference in the observed molecular weight of the isolated Flt4 ligand and the deduced molecular weight of the disclosed open reading frame of the Flt4 ligand sequence may then derive from sequences in the carboxyl terminal region of the latter. Also, the Flt4 ligand may be glycosylated at two putative N-linked glycosylation sites conforming to the consensus which can be identified in the deduced Flt4 ligand amino acid sequence (N-residues underlined in Fig. 10).

The carboxyl terminal amino acid sequences, which increase the predicted molecular weight of the Flt4 ligand subunit in comparison with other ligands of this family, show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring protein 3 (BRP3) sequence (Dignam and Case, Gene 88, 133-140, 1990), as depicted in Fig. 9A. Such a sequence may encode an independently folded domain present in a Flt4 ligand precursor and it may be involved, for example, in the regulation of secretion, solubility, stability, cell surface localization or activity of the Flt4 ligand. Interestingly, at least one cysteine motif of the BRP3 type is also found in the VEGF carboxy terminal amino acid sequences.

Thus, the Flt4-L mRNA may be first translated into a precursor from the mRNA corresponding to the Flt4-L clone, from which the mature ligand is derived by proteolytic cleavage. To define the mature Flt4 ligand product one first expresses the cDNA clone, which is deposited in the pcDNA1 expression vector, in cells, such as COS cells. One uses antibodies generated against Flt4-L-encoded peptides, such as amino terminal 23 amino acid peptide or bacterial Flt4 fusion proteins, such as a GST-fusion protein, to raise antibodies against the VEGF-homologous domain of Flt4 ligand. One then follows the biosynthesis and processing of the Flt4 ligand in the transfected cells by pulse-chase analysis using radioactive cysteine for labelling of the cells, immunoprecipitation and gel electrophoresis. Using antibodies against the two domains of the product of the Flt4-L clone material for radioactive or

PDGF/VEGF family of growth factors, as shown in Figure 10.

EXAMPLE 11

Stimulation of Flt4 autophosphorylation by the protein product of the Flt4 ligand vector

5 The 2.1 kb insert of the Flt4-L clone in pcDNA1 vector containing the open reading frame encoding the sequence shown in Fig: 9B- (SEQ ID NO: 32) was cut out from the vector using *HindIII* and *NotI* restriction enzymes, isolated from a preparative agarose gel and ligated to the corresponding sites in the pREP7 expression vector (Invitrogen). The pREP7
10 vector containing the above cloned insert was transfected into 293-EBNA cells (Invitrogen) using the calcium phosphate transfection method (Sambrook et al., Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989). About 48 hours after transfection the medium of the transfected cells was changed to DMEM medium lacking fetal calf serum and incubated
15 for 36 h. The thus conditioned medium was then collected, centrifuged at 5000 x g for 20 minutes, the supernatant was concentrated 5-fold using Centriprep 10 (Amicon) and used to stimulate NIH^{3T3} cells expressing LTRFlt41, as in Example 4. The cells were lysed, immunoprecipitated using
C anti-Flt4 antiserum and analysed by Western blotting using anti-
20 phosphotyrosine antibodies.

As can be seen from Fig. 11, lanes 1 and 3, the conditioned medium from two different dishes of the transfected cells stimulated Flt4 autophosphorylation in comparison with the medium from mock-transfected cells, which gave only background levels of phosphorylation of the Flt4
25 receptor (lane 2). When the concentrated conditioned medium was pre-absorbed with 20 μ l of a ^{slurry} of Flt4EC domain coupled to Sepharose (see
C example 4), no phosphorylation was obtained (lane 4), showing that the activity responsible for Flt4 autophosphorylation was indeed the Flt4 ligand. Thus, these results demonstrate that the Flt4-L plasmid vector clone having an
30 approximately 2.1 kb insert and containing the open reading frame shown in Fig. 9B is expressed into a Flt4 ligand in cells transfected with the Flt4-L expression vector clone, and thus is biologically active. The sequence encoded by that open reading frame is shown in SEQ ID NO: 33. Plasmid pFLT4-L has been deposited with the American Type Culture Collection, 12301

NO: 30) and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 31)
(sense-primer corresponding to nucleotides 2179-2199 of the pcDNAI vector).
The amplified product was subjected to digestion with *EcoRI* (Boehringer
Mannheim) to remove the portion of the DNA sequence amplified from the
5 pcDNAI vector and the resulting 153 bp fragment encoding the 5' end of the
Flt4 ligand was labeled with [³²P]-dCTP using the Klenow fragment of *E.coli*
DNA polymerase I (Boehringer Mannheim). That fragment was used as a
probe for hybridization screening of the amplified PC-3 cell cDNA library.

Filter replicas of the library were hybridized with the
10 radioactively labeled probe at 42 °C for 20 hours in a solution containing 50%
formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml
denatured salmon sperm DNA. Filters were washed twice in 1x SSC, 0.1%
SDS for 30 minutes at room temperature, then twice for 30 minutes at 65 °C
and exposed overnight.

15 On the basis of autoradiography, 10 positive recombinant
bacterial colonies hybridizing with the probe were chosen from the library.
C Plasmid DNA was purified from these colonies and analysed by *EcoRI* and
NotI digestion and agarose gel electrophoresis followed by ethidium bromide
staining. The ten plasmid clones were divided into three groups on the basis
20 of the presence of insert sizes of approximately 1.7, 1.9 and 2.1 kb,
respectively. Inserts of plasmids from each group were sequenced using the
T7 oligonucleotide as a primer and walking primers for subsequent sequencing
reactions.

Sequence analysis showed that all clones contain the open
25 reading frame encoding the NH2-terminal sequence of the Flt4 ligand.
Furthermore, the 2.1 and 1.9 kb clones also contained sequences encoding the
signal sequence (Fig. 9A, SS). The 5' end of the 1.7 kb clone began within
the signal sequence-encoding portion. Dideoxy sequencing was continued
using walking primers in the downstream direction. An 1140 nucleotide
30 portion of the sequence of the longest clone is shown in Figure 9B. As can be
seen in that figure, after the putative signal sequence the open reading frame
terminates in a TAA stop codon 318 amino acid residues further downstream
C from the ³³32 amino acid signal sequence. When compared with sequences in
the GenBank Database, the predicted protein product of this reading frame was
35 found to be homologous with the predicted amino acid sequences of the

The beginning of the sequence represents the pcDNAI vector and the underlined sequence represents the amplified product of the 5'-end of the insert. The ATG codon located upstream of that sequence in the same reading frame is followed by an open reading frame containing the amplified product of the putative signal sequence and the first 13 amino acid residues of the secreted Flt4 ligand. The cloning of the 5' end of the Flt4 cDNA, as described in the preceding two examples, is depicted schematically in Fig. 9A.

EXAMPLE 9

Amplification of the 3'-end of cDNA encoding the Flt4 ligand

Based upon the amplified 5'-sequence of the clones encoding the Flt4 ligand, two pairs of non-overlapping nested primers were designed to amplify the 3'-portion of the FLT4-L clones. The sense-strand primer 5'-ACAGAGAACAGGCCAACC-3' (SEQ ID NO: 26) and antisense-strand primer 5'-TCTAGCATTTAGGTGACAC-3' (SEQ ID NO: 27) corresponding to nucleotides 2311-2329 of the pcDNAI vector were used in a first "touchdown" PCR. The annealing temperature of the reaction was decreased 1°C every two cycles from 72°C to 52°C, at which temperature 15 additional cycles were carried out. The annealing time was 1 minute and extension at each cycle was carried out at 72°C for 3 minutes. DNA fragments of several sizes were obtained in the first amplification. Those products were diluted 1:200 in water and reamplified in PCR using the second pair of primers: 5'-AAGAGACTATAAAATTCGCTGCAGC-3' (SEQ ID NO: 28) and 5'-CCCTCTAGATGCATGCTCGA-3' (SEQ ID NO: 29) (antisense-strand primer corresponding to nucleotides 2279-2298 of the pcDNAI vector). Two DNA fragments were obtained, having sizes of 1350 bp and 570 bp. Those fragments were cloned into a pCRII vector and the inserts of the clones were sequenced. Both of these fragments were found to contain sequences encoding an amino acid sequence homologous to the VEGF sequence.

EXAMPLE 10

Screening the PC-3 cell cDNA library using the 5' PCR fragment of Flt4 ligand cDNA

A 219 bp 5'-terminal fragment of Flt4 ligand cDNA was amplified by PCR using the 5' PCR fragment described above and primers 5'-GTTGTAGTGTGCTGCAGCGAATTT-3' (antisense-strand primer, SEQ ID

PC-3 cDNA library. First, amplification was performed with primer 5'-TCNGTGGTTGTAGTGTGCTG-3' (SEQ ID NO: 19), which is the antisense-strand primer corresponding to amino acid residues 9-15 (AAHYNTE, SEQ ID NO: 20), and sense-strand primer 5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO: 21), corresponding to the T7 RNA promoter of the pcDNAI vector used for construction of the library. "Touchdown" PCR was used as disclosed in Don, *et al.*, *Nucl. Acids Res.*, 19: 4008 (1991), incorporated by reference herein. The annealing temperature of the two first cycles was 62 °C and subsequently the annealing temperature was decreased in every other cycle by 1 °C until a final temperature of 53 °C was reached, at which temperature 16 additional cycles were conducted. Annealing time was 1 minute and extension at each cycle was conducted at 72 °C for 1 minute. Multiple amplified DNA fragments were obtained in the first reaction. The products of the first amplification (1 ul of a 1:100 dilution in water) were used in the second amplification reaction employing the nested primers 5'-GTTGTAGTGTGCTGCAGCGAATTT-3' (SEQ ID NO: 22), an antisense-strand primer corresponding to amino acid residues 6-13 (KFAAAHYN, SEQ ID NO: 23) of the Flt4 ligand, and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 24), a sense-strand primer corresponding to nucleotides 2179-2199 of the pcDNAI vector. The sequences of these sense and antisense primers overlapped with the 3' ends of the corresponding primers used in the first PCR. "Touchdown" PCR was carried out by decreasing the annealing temperature from 72 °C to 66 °C and continuing with 18 additional cycles at 66 °C. The annealing time was 1 minute and extension at each cycle was carried out at 72 °C for 2 minutes. One major product of about 220 bp and three minor products of about 270 bp, 150 bp, and 100 bp were obtained.

The amplified fragment of approximately 220 bp was cut out from the agarose gel, cloned into a pCRII vector using the TA cloning kit (Invitrogen) and sequenced. Three recombinant clones were ^{analysed} and they contained the sequence 5'-

TCACTATAGGGAGACCCAAGCTTGGTACCGAGCTCGGATCCACTAGT
AACGGCCGCCAGTGTGGTGGGAATTCGACGAACTCATGACTGTACTCT
ACCCAGAATATTGGAAAATGTACAAGTGTGCTAGCTAAGGCAAGGAGGC
TGGCAACATAACAGAGAACAGGCCAACCTCAACTCAAGGACAGAAG
AGACTATAAAATTCGCTGCAGCACACTACAAC- 3' (SEQ ID NO: 25).

lysates were centrifuged for 20 minutes at 15,000 x g. The supernatants were incubated for 2 hours on ice with 3 ul of the antiserum against the Flt4 C-terminus described in Example 2 and also in Pajusola, *et al. Oncogene* 8: 2931-2937, (1993), incorporated by reference herein.

5 After a 2 hour incubation in the presence of anti-Flt4 antiserum, protein A-Sepharose (Pharmacia) was added and incubation was continued for 45 minutes with rotation. The immunoprecipitates were washed three times with the immunoprecipitation buffer and twice with 10 mM Tris, pH7.5 before analysis in SDS-PAGE. Polypeptides were transferred to nitrocellulose and
10 analyzed by Western blotting using Flt4- or phosphotyrosine-specific antisera and the ECL method (Amersham International, Buckinghamshire, England). Anti-phosphotyrosine monoclonal antibodies (anti-PTyr; PY20) were purchased from Transduction Laboratories (Lexington, Kentucky). In some cases, the filters were restained with a second antibody after stripping. The stripping of
15 the filters was done for 30 minutes at 50°C in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7 with occasional agitation.

As shown in Figure 4, the PC-3 cell conditioned medium stimulated tyrosine phosphorylation of a 125 kD polypeptide when Flt4-
c expressing NIH3T3³⁷³ cells were treated with the indicated preparations of
20 media, lysed, and the lysates were immunoprecipitated with anti-Flt4 antiserum followed by SDS-PAGE, Western blotting, and staining using anti-PTyr antibodies. The resulting band was weakly phosphorylated upon stimulation with unconcentrated PC-3 conditioned medium (lane 2). The 125 kD band comigrated with the tyrosine phosphorylated, processed form of the
25 mature Flt4 from pervanadate-treated cells (compare lanes 2 and 7 of Fig. 4, see also Figure 5A). Comigration was confirmed upon restaining with anti-Flt4 antibodies as is also shown in Figure 5A (panel on the right). In order to show that the 125 kD polypeptide is not a non-specific component of the conditioned medium reactive with anti-phosphotyrosine antibodies, 15 ul of
c 30 conditioned medium ^{were} separated by SDS-PAGE, blotted on nitrocellulose and the blot was stained with anti-PTyr antibodies. No signal was obtained (Fig. 5B). Also, unconditioned medium failed to stimulate Flt4 phosphorylation, as shown in Figure 4, lane 1.

As shown in Figure 4, lane 3, stimulating activity was
35 considerably increased when the PC-3 conditioned medium was concentrated

above (the *HindIII* site is in the 5' junction of the Flt4 insert with the pLTRpoly portion of the vector, the *SphI* site is in Flt4 cDNA). The resultant Flt4EC insert was then ligated as a *BamHI* fragment into the *BamHI* site in the pVTBac plasmid as disclosed in Tessier *et al.*, *Gene* 98: 177-183 (1991),
5 incorporated by reference herein. The orientation was confirmed to be correct by partial sequencing so that the open reading frame of the signal sequence-encoding portion of the vector continued in frame with the Flt4 sequence. That construct was transfected together with the baculovirus genomic DNA into SF-9 cells by lipofection. Recombinant virus was purified, amplified and
10 used for infection of High-Five cells (Invitrogen, San Diego, CA) using methods standard in the art. The Flt4 extracellular domain (Flt4EC) was purified from the culture medium of the infected High-Five cells using Ni-NTA affinity chromatography according to manufacturer's instructions (Qiagen) for binding and elution of the 6xHis tag encoded in the COOH-
15 terminus of the recombinant Flt4 extracellular domain.

EXAMPLE 4

Isolation of Flt4 Ligand from Conditioned Media

An Flt4 ligand according to the invention was isolated from conditioned media from PC-3 prostatic adenocarcinoma cell line CRL1435
20 from the American Type Culture Collection and cultured as instructed by the supplier in Ham's F-12 Nutrient mixture (GIBCO) containing 7% fetal calf serum. In order to prepare the conditioned media, confluent PC-3 cells were cultured for 7 days in Ham's F-12 Nutrient mixture (GIBCO) in the absence of fetal bovine serum. Medium was then cleared by centrifugation at 10,000 g
25 for 20 minutes. The medium was then screened to determine its ability to induce tyrosine phosphorylation of Flt4 by exposure to NIH^{3T3} cells which had been transfected with Flt4-encoding cDNA using the pLTRFlt4l vector.
C For receptor stimulation experiments, subconfluent NIH^{3T3} cells were starved overnight in serum-free DMEM medium (GIBCO) containing 0.2% BSA. The
30 cells were stimulated with the conditioned media for 5 minutes, washed twice with cold PBS containing 100 μ M vanadate and lysed in RIPA buffer (10 mM Tris pH 7.5, 50 mM NaCl, 0.5% sodium deoxycholate, 0.5% Nonidet P40 (BDH, Poole, England), 0.1% SDS, 0.1 U/ml Aprotinin (Boehringer Mannheim), 1 mM vanadate) for receptor immunoprecipitation analysis. The

SphI fragment of the S2.5 plasmid. The resulting vector was digested with *EcoRI* and *Clal* and ligated to a 138 bp PCR fragment amplified from the 0.6 kb *EcoRI* fragment (base pairs 3789 to 4416 in the Genbank X68203 sequence) which encodes the 3' end of Flt4s shown in Figure 1 of Pajusola *et al.*,

- 5 *Cancer Res.* 52:5738-5743, 1992, using the oligonucleotides 5'-
CGGAATTCCCC CATGACCCCA AC-3' (SEQ ID NO: 4) (forward, *EcoRI*
site underlined) and 5'-CCATCGATGG ATCCTACCTG AAGCCGCTTT
CTT-3' (SEQ ID NO: 5) (reverse, *Clal* site underlined). The coding domain
was completed by ligation of the 1.2 kb *EcoRI* fragment (base pairs 2535-3789
10 of sequence X68203) into the above construct. The complete cDNA was
subcloned as a *HindIII-Clal*(blunted) fragment (this *Clal* site was also included
in the 3' primer used to construct the 3' end of the coding sequence) to the
pLTRpoly expression vector reported in Mäkelä *et al.*, *Gene*, 118: 293-294
(1992) (Genbank accession number X60280), incorporated by reference herein,
15 using its *HindIII-Acc I*(blunted) restriction sites.

- The long form of Flt4 was produced by replacing the 3'-end of
the short form as follows: The 3' region of the Flt4l cDNA was PCR-
amplified using a gene specific and a pGEM 3Z vector specific (SP6 promoter)
oligonucleotide 5'-ATTTAGGTGACACTATA-3' (SEQ ID NO: 6) as reverse
20 and forward primers, respectively, and an Flt4l cDNA clone containing a 495
bp *EcoRI* fragment extending downstream of the *EcoRI* site at nucleotide 3789
of the Genbank X68203 sequence (the sequence downstream of this *EcoRI* site
is deposited as the Flt4 long form 3' sequence having Genbank accession
number S66407). The gene specific oligonucleotide contained a *BamHI*
25 restriction site located right after the end of the coding region. The sequence
of that (reverse primer) oligonucleotide was 5'-
CCATCGATGGATCCCGATGCTGCTTAGTAGCTGT-3' (SEQ ID NO: 7)
(*BamHI* site is underlined). The PCR product was digested with *EcoRI* and
BamHI and transferred in frame to LTRFlt4s vector fragment from which the
30 coding sequences downstream of the *EcoRI* site at base pair 2535 (see
sequence X68203) had been removed by *EcoRI-BamHI* digestion. Again, the
coding domain was completed by ligation of the 1.2 kb *EcoRI* fragment (base
pairs 2535-3789 of sequence X68203) back into the resulting construct.

containing base pairs 56-2534 of the Flt4s into the *EcoRI* site of the pSP73 vector (Promega, Madison, WI).

Since cDNA libraries used for screening of Flt4 cDNAs did not contain its most 5' protein-coding sequences, inverse PCR was used for the amplification of the 5' end of Flt4 corresponding to the first 12 amino acid residues (MQRGAALCLRLW). Poly(A)⁺ RNA was isolated from the HEL cells and double-stranded cDNA copy was synthesized using the Amersham cDNA Synthesis System Plus kit and a gene specific primer: 5'-TGTCCTCGCTGTCCTTGTCT-3' (SEQ ID NO: 1), which was located 195 bp downstream of the 5' end of clone S2.5. Double stranded cDNA was treated with T4 DNA polymerase to blunt the ends and cDNA was purified with Centricon 100 ^{ultra} (Amicon Inc., Beverly, MA). Circularization was made in a total volume of 150 ul. The reaction mixture contained ligation buffer, 5% PEG-8000, 1 mM DTT and 8U of T4 DNA ligase (New England Biolabs). Ligation was carried out at 16°C for 16 hours. Fifteen μ l of this reaction mix was used in a standard 100 ul PCR reaction containing 100 ng of specific primers including *SacI* and *PstI* restriction sites, present in this segment of the Flt4 cDNA, and 1 unit of Taq DNA polymerase (Perkin Elmer Cetus). Two rounds of PCR were performed using 33 cycles (denaturation at 95°C for 1 minute, annealing at 55°C for 2 minutes and elongation at 72°C for 4 minutes). The PCR mixture was treated sequentially with the *SacI* and *PstI* restriction enzymes and after purification with MagicPCR Preps (Promega) DNA fragments were subcloned into the pGEM3Zf(+) vector for sequencing. The sequence obtained corresponds to the 5' end of the Flt4s cDNA clone deposited in the Genbank Database as Accession No. X68203.

The sequence encoding the first 12 amino acid residues was added to the expression construct by ligating an *SphI* digested PCR fragment amplified using reverse transcription-PCR of poly(A)⁺ RNA isolated from the HEL cells using the oligonucleotides 5'-ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG GACTCCTGGA-3' (SEQ ID NO: 2)(forward primer, *SphI* site underlined, the translational start codon marked in bold follows an optimized Kozak consensus sequence Kozak, *Nucl. Acids Res.* 15: 8125-8148, 1987) and 5'-ACATGCATGC CCCGCCGGT CATCC-3' (SEQ ID NO: 3) (reverse primer, *SphI* site underlined) to the 5' end of the S2.5 fragment, thus replacing unique

and VEGF-C thus increase our understanding of the complexity of the specific and redundant positive signals for endothelial cells involved in vasculogenesis, angiogenesis, permeability and perhaps also other endothelial functions.

C Also described herein is the localization of the VEGF-C gene
5 in human chromosomes by analysis of somatic cell hybrids and fluorescence *in situ* hybridization (FISH). Southern blotting and polymerase chain reaction analysis of somatic cell hybrids and fluorescence *in situ* hybridization of metaphase chromosomes was used to assess the chromosomal localization of the VEGF-C gene. The VEGF-C gene was located on chromosome 4q34,
10 close to the human aspartylglucosaminidase gene previously mapped to 4q34-35. The VEGF-C locus in 4q34 is a candidate target for mutations leading to vascular malformations or cardiovascular diseases. Expression studies by Northern blotting and hybridization show abundant VEGF-C expression in heart and skeletal muscle; other tissues, such as lung and kidney,
C 15 also express these genes. Whereas PlGF is predominantly expressed in the placenta, the expression patterns of the three VEGFs overlap in many tissues, which suggests that they may form heterodimers and interact to exert their physiological functions.

C 20 Targeted mutagenesis leading to inactivation of the VEGF receptor loci in the mouse genome have shown that VEGFR-1 is necessary for the proper organization of endothelial cells forming the vascular endothelium, while VEGFR-2 is necessary for the generation of both endothelial and hematopoietic cells. This suggests that the four genes of the VEGF family can be targets for mutations leading to vascular malformations or cardiovascular
25 diseases.

The following Examples illustrate preferred embodiments of the invention, wherein the isolation, characterization, and function of Flt4 ligands and ligand-encoding nucleic acids according to the invention are shown.

EXAMPLE 1

30 Production of pLTR-Flt4l expression vector

Construction of the LTR-Flt4l vector is schematically shown in Figure 2. The full-length Flt4s cDNA (Genbank Accession No. X68203) was assembled by first subcloning the S2.5 fragment, reported in Pajusola *et al.*, *Cancer Res.* 52:5738-5743 (1992), incorporated by reference herein,

herein further suggests that this gene product also is involved in the maintenance of the differentiated functions of the lymphatic endothelium where VEGFR-3 is expressed (Kaipainen et al., 1995). Lymphatic capillaries do not have well formed basal laminae and an interesting possibility remains that the silk-like BR3P motif is involved in producing a supramolecular structure which could regulate the availability of VEGF-C in tissues. However, as shown here, VEGF-C also activates VEGFR-2, which is abundant in proliferating endothelial cells of vascular sprouts and branching vessels of embryonic tissues, but decreased in adult tissues. Millauer et al., *Nature*, 367:576-78 (1993). These data have suggested that VEGFR-2 is a major regulator of vasculogenesis and angiogenesis. VEGF-C may thus have a unique effect in lymphatic endothelium and a more redundant function shared with VEGF in angiogenesis and possibly permeability regulation of several types of endothelia. Because VEGF-C stimulates the VEGFR-2 and promotes endothelial migration, a utility for VEGF-C is suggested as an inducer of angiogenesis of blood and lymphatic vessels in wound healing, tissue transplantation, in eye ^{diseases}, in the formation of collateral vessels to around arterial stenoses and into injured tissues after infarction.

Taken together, these results show an increased complexity of signalling in the vascular endothelium. They reinforce the concept that when organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases in several types of functionally and morphologically distinct vessels. However, upon suitable angiogenic stimuli, endothelial cells can re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their tissue environment. This process of angiogenesis concurrent with tissue development and regeneration depends on the tightly controlled balance between positive and negative signals for endothelial cell proliferation, migration, differentiation and survival. Previously-identified growth factors promoting angiogenesis include the fibroblast growth factors, hepatocyte growth factor/scatter factor, PDGF and TGF α . (See, e.g., Folkman, *Nature Med.* 1:27-31 (1995); Friesel and Maciag, *FASEB J.* 9:919-25 (1995); Mustonen and Alitalo, *J. Cell Biol.*, 129:895-98 (1995). However, VEGF has been the only growth factor relatively specific for endothelial cells. The newly identified factors VEGF-B

Mutational analysis of the cysteine residues involved in the interchain disulfide bridges ^{has} have shown that, in contrast to PDGF, VEGF dimers need to be held together by these covalent interactions in order to maintain biological activity. Disulfide linking of the VEGF-C polypeptide chain was evident in the analysis of VEGF-C in nonreducing conditions.

VEGFR-3, which thus distinguishes between VEGF and VEGF-C, is closely related ⁱⁿ to structure to VEGFR-1 and VEGFR-2. Finnerty, *et al.*, *Oncogene*, 8:2293-98 (1993); Galland, *et al.*, *Oncogene*, 8:1233-40 (1993); Pajusola, *et al.*, *Cancer Res.*, 52:5738-43 (1992). However, the mature form of VEGFR-3 differs from the two other VEGFRs in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola, *et al.*, *Oncogene*, 9:3545-55 (1994). Another difference is that the 4.5 and 5.8 kb VEGFR-3 mRNAs encode polypeptides differing in their C-termini and apparently in their signalling properties due to the use of alternative 3' exons. Borg *et al.*, *Oncogene*, 10:973-84 (1995); Pajusola *et al.*, *Oncogene*, 8:2931-37 (1993).

Besides VEGFR-3, VEGFR-2 tyrosine kinase also is activated in response to VEGF-C. VEGFR-2 mediated signals cause striking changes in the morphology, actin reorganization and membrane ruffling of porcine aortic endothelial cells overexpressing this receptor. In these cells, VEGFR-2 also mediated ligand-induced chemotaxis and mitogenicity. Waltenberger *et al.*, *J. Biol. Chem.*, 269:26988-95 (1994). Similarly, the receptor chimera CSF-1R/VEGFR-3 was mitogenic when ectopically expressed in NIH3T3 ^{3T3} fibroblastic cells, but not in porcine aortic endothelial cells (Pajusola *et al.*, 1994). Consistent with such results, the bovine capillary endothelial cells ^(BCE) which express VEGFR-2 mRNA but very little or no VEGFR-1 or VEGFR-3 mRNAs, showed enhanced migration when stimulated with VEGF-C. As shown here, light microscopy of the BCE cell cultures in collagen gel also suggested that VEGF-C stimulated the proliferation of these cells. The already existing data thus indicate that the VEGF ligands and receptors show a great specificity in their signalling, which may be cell type dependent.

The expression pattern of the VEGFR-3 (Kaipainen *et al.*, *Proc. Natl. Acad. Sci. USA*, 92:3566-70 (1995)) suggests that VEGF-C may function in the formation of the venous and lymphatic vascular systems during embryogenesis. Constitutive expression of VEGF-C in adult tissues shown

latter. Proteolytic processing of the VEGF-C precursor may occur at more than one cleavage site because the 32 kD molecular mass of the recombinant secreted ligand was also less than the deduced molecular mass of VEGF-C ORF without the signal peptide. By extrapolation from studies of the structure of PDGF (Heldin, *et al.*, *Growth Factors*, 8:245-52 (1993)), one can speculate that the region critical for receptor binding and activation by VEGF-C is contained within the amino-terminal first 180 or so amino acid residues of the secreted VEGF-C protein lacking the signal sequence. In fact, the region critical for receptor binding and activation by VEGF-C is believed to be contained within the first approximately 120 amino acid residues of the secreted VEGF-C protein lacking the signal sequence. Thus, the 23 kD polypeptide binding VEGFR-3 is likely to represent the VEGF-homologous domain. After biosynthesis, the nascent VEGF-C polypeptide may be glycosylated at three putative N-linked glycosylation sites identified in the deduced VEGF-C amino acid sequence.

The carboxyl terminal amino acid sequences, which increase the length of the VEGF-C polypeptide in comparison with other ligands of this family, show a pattern of spacing of cysteine residues reminiscent of the ^{Bellini} ~~Bellini~~ ring 3 protein (BR3P) sequence (Dignam and Case, *Gene*, 88:133-40 (1990); Paulsson, *et al.*, *J. Mol. Biol.*, 211:331-49 (1990)). This novel C-terminal silk protein-like structural motif of VEGF-C may fold into an independent domain, which, on the basis of the considerations above, is at least partially cleaved off after biosynthesis. Interestingly, at least one cysteine motif of the BR3P type is also found in the carboxyl terminus of VEGF. In our experiments both the putative precursor and cleaved ligand were detected in the cell culture media, although processing was apparently cell-associated on the basis of the pulse-chase experiments. The determination of the amino terminal sequence of the isolated carboxyl terminal fragment will allow the identification of the proteolytic processing site. The generation of antibodies against different parts of the VEGF-C molecule will allow the exact determination of the precursor-product relationship and ratio, their cellular distribution, and the kinetics of processing and secretion.

VEGF-C has a conserved pattern of eight cysteine residues, which may participate in the formation of intra- and interchain disulfide bonds, creating an antiparallel dimeric biologically active molecule, similar to PDGF.

Ligands of the
C which are ligands for the Flt4 receptor tyrosine kinase (VEGFR-3). ~~Claimed~~
C ^{invention} ligands are members of a family of platelet-derived growth factors/vascular
endothelial growth factors which promote mitosis and proliferation of vascular
endothelial cells and/or mesodermal cells. Ligands recognizing the Flt4
5 receptor tyrosine kinase were purified from a PC-3 prostatic adenocarcinoma
cell line (ATCC CRL1435). When applied to a population of cells expressing
the Flt4 receptor, ligands of the invention stimulate autophosphorylation,
resulting in receptor activation. The invention also provides inhibitors of the
Flt4 receptor, including antibodies directed against the ligand. A ligand
10 according to the invention may be coexpressed as a larger precursor which is
cleaved to produce the ligand. A coexpressed region in some cases results
from alternative splicing of RNA of the ligand gene. Such a co-expressed
region may be a function of the particular expression system used to obtain the
ligand. The skilled artisan understands that in recombinant production of
15 proteins, additional sequence may be expressed along with a functional peptide
depending upon the particular recombinant construct used to express the
protein, and subsequently removed to obtain the desired ligand. In some cases
the recombinant ligand can be made lacking certain residues of the
endogenous/natural ligand. Moreover, it is well-known in that conservative
20 replacements may be made in a protein which do not alter the function of the
protein. Accordingly, it is anticipated that such alterations are within the
scope of the claims. It is intended that the precursor sequence shown in SEQ
ID NO: 33 is capable of stimulating the Flt4 ligand without any further
processing in a manner similar to that in which VEGF stimulates its receptor
25 in its unprocessed form.

Results reported herein show that VEGFR-3 transmits signals
for a novel growth factor. This conclusion is based on the specific binding of
VEGF-C to recombinant Flt4EC (Flt4 extracellular domain) protein and the
induction of VEGFR-3 autophosphorylation by medium from VEGF-C
30 transfected cells. In contrast, VEGF and PlGF did not show specific binding
to VEGFR-3 or induce its autophosphorylation.

A major part of the difference in the observed molecular mass
of the purified and recombinant VEGF-C and the deduced molecular mass of
the VEGF-C encoded by the VEGF-C open reading frame (ORF) may be due
35 to proteolytic removal of sequences in the carboxyl terminal region of the

Figure 11 shows the stimulation of autophosphorylation of the Flt4 receptor by conditioned medium from cells transfected with the Flt4-L (VEGF-C) expression vector.

C 5 Figure 12 shows Northern blotting analysis of Flt4-L (VEGF-C) mRNA in tumor cell lines ^{and in brain tissue}.

C Figure 13A is an autoradiograph showing recombinant ^{VEGF-C} ~~VEGF-C~~ isolated following a pulse-chase experiment and electrophoresed via SDS-PAGE under reducing conditions.

C 10 Figure 13B is a photograph of polyacrylamide gel showing that recombinant ^{VEGF-C} ~~VEGF-C~~ forms are disulfide-linked in nonreducing conditions.

Figure 14A and 14B depict Western blots showing that VEGF-C stimulates autophosphorylation of VEGFR-2 (KDR) but has no effect on PDGFR- β phosphorylation.

15 Figure 15A and 15B show that VEGF-C stimulates endothelial cell migration in a three-dimensional collagen gel assay.

Figure 16A shows the expression of VEGF-C mRNA in human adult tissues.

Figure 16B shows the expression of VEGF, VEGF-B, and VEGF-C in selected human fetal tissues.

20 Figure 17 schematically depicts the chromosomal localization of the VEGF-C gene.

Figure 18 is a Northern blot hybridization study showing the effects of hypoxia on the mRNA expression of VEGF-A, VEGF-B and VEGF-C.

25 DETAILED DESCRIPTION OF THE INVENTION

C Described herein is the isolation of a novel vascular endothelial growth factor ^{the cloning of a cDNA encoding this growth factor} and its cloning from a cDNA library prepared from the human prostatic adenocarcinoma cell line PC-3. The isolated cDNA encodes a protein which is proteolytically processed and secreted to cell culture medium.

30 The secreted protein, designated VEGF-C, binds to the extracellular domain of Flt4 (designated VEGFR-3) and induces tyrosine autophosphorylation of Flt4 and VEGFR-2. VEGF-C also stimulates the migration of endothelial cells in collagen gel.

The present invention also is directed to novel growth factors

oligonucleotides, and peptides which block the Flt4 receptor, all of which are intended as aspects of the invention.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a schematic diagram showing major endothelial cell
5 receptor tyrosine kinases and growth factors involved in vasculogenesis and angiogenesis.

Figure 2 schematically depicts the construction of the pLTRFlt4l expression vector.

Figure 3 schematically depicts the construction of the
10 baculovirus vector encoding a secreted soluble Flt4 extracellular domain (Flt4EC).

Figure 4 shows results of stimulation of Flt4 autophosphorylation by conditioned medium from PC-3 cell cultures.
C *Figures 5A, 5B, and 5C show*
15 polypeptide of Flt4-transfected cells stimulated with PC-3 conditioned medium is the 125 kD Flt4 polypeptide (VEGFR-3).

Figure 6 shows Western analysis of the Flt4 ligand activity isolated from PC-3 conditioned medium.

C *chromatographic fractions from*
C 20 *no affinity purification* Figure 7 shows results of gel electrophoresis of fractions from the Western analysis of Flt4 ligand (VEGF-C) isolated from PC-3 conditioned medium.

Figure 8 shows results of Western analysis of Flt4 autophosphorylation induced by either the Flt4 ligand (VEGF-C), VEGF, or PIGF.

25 Figure 9A schematically depicts the cloning and analysis of the Flt4 ligand, VEGF-C. The VEGF-C coding sequence (shaded boxes) and signal sequence (ss) are depicted between 5' and 3' untranslated (ut) nucleic acid regions.

C
30 Figure 9B shows the nucleotide and deduced amino acid sequence of the coding portion of Flt4 ligand cDNA. The cleavage site for the putative signal peptide is indicated with a shaded triangle.

Figure 10 shows a comparison of the deduced amino acid sequences of PDGF-A, -B, two PIGF isoforms, four VEGF isoforms and Flt4 ligand (VEGF-C).
C
C1

In another aspect, the invention includes an antibody which is specifically reactive with polypeptides of the invention. Antibodies, both monoclonal and polyclonal, may be made against a ligand of the invention according to standard techniques in the art. Such antibodies may be used in diagnostic applications to monitor angiogenesis, vascularization, lymphatic vessels and their disease states, wound healing, or certain hematopoietic or leukemia cells, or they may be used to block or activate the Flt4 receptor.

Ligands according to the invention may be labeled with a detectable label and used to identify their corresponding receptors *in situ*. Labeled Flt4 ligand and anti-Flt4 ligand antibodies may be used as imaging agents in the detection of lymphatic vessels, high endothelial venules, and Flt4 receptors expressed in histochemical tissue sections. The ligand or antibody may be covalently or non-covalently coupled to a suitable supermagnetic, paramagnetic, electron dense, echogenic, or radioactive agent for imaging. Other, non-radioactive labels, such as biotin and avidin, may also be used.

The present invention also provides diagnostic and clinical applications for claimed ligands. In a preferred embodiment, Flt4 ligands or precursors are used to accelerate angiogenesis, *e.g.*, during wound healing, or to promote the endothelial functions of lymphatic vessels. Ligands may be applied in any suitable manner using an appropriate pharmaceutically-acceptable vehicle. Ligands also may be used to quantify future metastatic risk by assaying biopsy material for the presence of active receptors or ligands in a binding assay or kit using detectably-labeled ligand. An Flt4 ligand according to the invention also may be used to promote re-growth or permeability of lymphatic vessels in, for example, organ transplant patients. Ligands according to the invention also may be used to treat or prevent inflammation, edema, aplasia of the lymphatic vessels, lymphatic obstruction, elephantiasis, and Milroy's disease. Finally, Flt4 ligands may be used to stimulate lymphocyte production and maturation, and to promote or inhibit trafficking of leukocytes between tissues and lymphatic vessels or to affect migration in and out of the thymus.

Inhibitors of the Flt4 ligand may be used to control endothelial cell proliferation and lymphangiomas. For example, such inhibitors may be used to arrest metastatic growth or spread, or to control other aspects of endothelial cell expression and growth. Inhibitors include antibodies, antisense

comprises approximately amino acids 1-120 of SEQ ID NO: 33. Another preferred polypeptide of the invention comprises approximately amino acids 1-180 of SEQ ID NO: 33.

5 The present invention also provides a cDNA encoding a novel polypeptide, designated VEGF-C, that is structurally homologous to VEGF. VEGF-C is a ligand for the FLT4 receptor tyrosine kinase (VEGFR-3), a receptor tyrosine kinase related to VEGFR-1 and VEGFR-2 that does not bind VEGF. VEGFR-3 is expressed in venous and lymphatic endothelia of fetal tissues and predominantly in lymphatic endothelial of adult tissues. Kaipainen
10 et al., *Cancer Res.*, 54:6571-77 (1994); Kaipainen et al., *Proc. Natl. Acad. Sci. USA*, 92:3566-70 (1995).

Thus, in a preferred embodiment, the invention includes a purified and isolated nucleic acid (e.g., a DNA or an RNA) encoding an Flt4 ligand precursor. Due to the degeneracy of the genetic code, numerous such
15 coding sequences are possible, each having in common the coding of the amino acid sequence shown in SEQ ID NO: 33. As set forth above, the invention includes polypeptides which comprise a portion of the amino acid sequence shown in SEQ ID NO: 33 and which bind the Flt4 receptor tyrosine kinase (herein designated VEGFR-3); the invention also is intended to include
20 nucleic acids encoding these polypeptides. Ligand precursors according to the invention, when expressed in an appropriate host cell, produce, via cleavage, a peptide which binds specifically to the Flt4 receptor tyrosine kinase (VEGFR-3). The nucleotide sequence shown in SEQ ID NO:32 contains a preferred nucleotide sequence encoding the Flt4 ligand (VEGF-C).

25 The present invention also provides a cell line which produces an Flt4 ligand. The ligand may be purified and isolated directly from the cell culture medium. Also provided are vectors comprising a DNA encoding the Flt4 ligand, and host cells comprising the vectors. Preferred vectors of the invention are capable of expressing the Flt4 ligand under the control of
30 appropriate promoters and other control sequences. A preferred vector of the invention is plasmid pFLT4-L, having ATCC accession no. ⁷⁷²³¹27321.

The invention further includes a method of making polypeptides
of the invention. In a preferred method, a nucleic acid or vector of the invention is expressed in a host cell, and a polypeptide of the invention is
35 purified from the host cell or the host cell growth medium.

and VEGFR-1 also binds the related placenta growth factor (PlGF; Kd about 200 pM), while the ligands for Tie, Tek, and Flt4 have not yet been reported.

SUMMARY OF THE INVENTION

The present invention provides a ligand for the Flt4 receptor tyrosine kinase. Thus, the invention provides a purified and isolated polypeptide which specifically binds to the Flt4 receptor tyrosine kinase. In a preferred embodiment, the ligand comprises a fragment of the amino acid sequence shown in SEQ ID NO: 33 which specifically binds to the Flt4 receptor tyrosine kinase.

The present invention also provides a precursor of an Flt4 ligand, wherein the precursor comprises the amino acid sequence shown in SEQ ID NO: 33. Thus, the invention includes a purified and isolated polypeptide having the amino acid sequence shown in SEQ ID NO: 33.

A putative ³³ amino acid signal peptide has been identified in the amino acid sequence shown in SEQ ID NO: 33. Thus, in a related aspect, the invention includes a purified and isolated polypeptide comprising amino acids 1-³¹⁷ of SEQ ID NO: 33. The Flt4 ligand precursor is proteolytically cleaved upon expression to produce an approximately 23 kD peptide which is the Flt4 ligand (herein designated VEGF-C). Thus, the invention includes a polypeptide having an amino acid sequence comprising a portion of SEQ ID NO: ³³3, the portion encoding a fragment capable of specifically binding to Flt4. A preferred fragment has a molecular weight of about 23 kDa as assessed by SDS-PAGE under reducing conditions. In a preferred embodiment of the invention, an Flt4 ligand is provided which is the cleavage product of the precursor peptide shown in SEQ ID NO: 33 and which has a molecular weight of approximately 23 kD under reducing conditions.

Evidence suggests that the amino acids essential for retaining Flt4 ligand activity are contained within approximately amino acids 1-120 of SEQ ID NO: 33, and that the proteolytic cleavage to produce a mature, naturally-occurring Flt4 ligand occurs within approximately amino acids 1-180 of SEQ ID NO: 33. Accordingly, ^{polypeptides} preferred polypeptides of the invention include polypeptides comprising amino acids 1-120, 1-121, 1-122, 1-123, 1-124 ... 1-178, 1-179, and 1-180 of SEQ ID NO: 33, wherein said polypeptides specifically bind to an Flt4 receptor tyrosine kinase. A preferred Flt4 ligand

rather than five immunoglobulin-like loops in their extracellular domain and they possess a longer kinase insert than normally observed in this family. The expression of VEGF receptors occurs mainly in vascular endothelial cells, although some may be present on monocytes and melanoma cells. Only
5 endothelial cells have been reported to proliferate in response to VEGF, and endothelial cells from different sources show different responses. Thus, the signals mediated through VEGFR-1 and VEGFR-2 appear to be cell type specific.

The Flt4 receptor tyrosine kinase (VEGFR-3) is closely related
10 in structure to the products of the VEGFR-1 and VEGFR-2 genes. Despite this similarity, the mature form of Flt4 differs from the VEGF receptors in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola *et al.*, *Cancer Res.*, 52:5738-5743 (1992). The
C 4,5 and 5.8 kb ^{Flt4} ~~Flt4~~ mRNAs encode polypeptides which differ in their C-
15 termini due to the use of alternative 3' exons. The VEGFs do not show specific binding to Flt4 or induce its autophosphorylation.

Expression of Flt4 appears to be more restricted than expression of VEGFR-1 or VEGFR-2. The expression of Flt4 first becomes detectable by
20 *in situ* hybridization in the angioblasts of head mesenchyme, the cardinal vein, and extraembryonically in the allantois of 8.5 day p.c. mouse embryos. In
C 12.5 day p.c. embryos the ^{Flt4} ~~Flt4~~ signal is observed in developing venous and presumptive lymphatic endothelia, but arterial endothelia appear negative. During later stages of development, Flt4 mRNA becomes restricted to
25 developing lymphatic vessels. Only the lymphatic endothelia and some high endothelial venules express Flt4 mRNA in adult human tissues and increased expression occurs in lymphatic sinuses in metastatic lymph nodes and in lymphangioma. These results support the theory of the venous origin of lymphatic vessels.

Five endothelial cell specific receptor tyrosine kinases, Flt-1
30 (VEGFR-1), KDR/Flk-1 (VEGFR-2), Flt4, Tie and Tek/Tie-2 have so far been described, which possess the intrinsic tyrosine kinase activity essential for signal transduction. Targeted mutations inactivating Flt-1, Flk-1, Tie and Tek
in mouse embryos have indicated their essential and specific roles in
35 vasculogenesis and angiogenesis at the molecular level. VEGFR-1 and VEGFR-2 bind VEGF with high affinity (Kd 16 pM and 760 pM, respectively)

is a dimeric glycoprotein of disulfide-linked 23 kDa subunits. Other reported effects of VEGF include the mobilization of intracellular calcium, the induction of plasminogen activator and plasminogen activator inhibitor-1 synthesis, stimulation of hexose transport in endothelial cells, and promotion of monocyte migration *in vitro*. Four VEGF isoforms, encoded by distinct mRNA splice variants, appear to be equally capable of stimulating mitogenesis in endothelial cells. However, each isoform has a different affinity for cell surface proteoglycans, which behave as low affinity receptors for VEGF. The 121 and 165 amino acid isoforms of VEGF are secreted in a soluble form, whereas the isoforms of 189 and 206 amino acid residues remain cell surface associated and have a strong affinity for heparin.

VEGF was originally purified from several sources on the basis of its mitogenic activity toward endothelial cells, and also by its ability to induce microvascular permeability, hence it is also called vascular permeability factor (VPF). VEGF produces signals through two receptor tyrosine kinases, VEGFR-1 (FLT-1) and VEGFR-2 (KDR/Flk-1), which are expressed specifically on endothelial cells. The VEGF-related placenta growth factor (PlGF) was recently shown to bind to VEGFR-1 with high affinity. PlGF was able to enhance the growth factor activity of VEGF, but it did not stimulate endothelial cells on its own. Naturally occurring VEGF/PlGF heterodimers were nearly as potent mitogens as VEGF homodimers for endothelial cells.

The pattern of VEGF expression suggests its involvement in the development and maintenance of the normal vascular system and in tumor angiogenesis. During murine development, the entire 7.5 day post-coital (p.c.) endoderm expresses VEGF and the ventricular neuroectoderm produces VEGF at the capillary ingrowth stage. See Breier, *et al.*, *Development*, 114:521-523 (1992). On day two of quail development, the vascularized area of the yolk sac as well as the whole embryo show expression of VEGF. In addition, epithelial cells next to fenestrated endothelia in adult mice show persistent VEGF expression, suggesting a role in the maintenance of this specific endothelial phenotype and function.

Two high affinity receptors for VEGF have been characterized. These are VEGFR-1/Flt-1 (fms-like tyrosine kinase-1) and VEGFR-2/Kdr/Flk-1 (kinase insert domain containing receptor/fetal liver kinase-1). Those receptors are classified in the PDGF-receptor family, but they have seven

Key signals regulating cell growth and differentiation are mediated by polypeptide growth factors and their transmembrane receptors, many of which are tyrosine kinases. Autophosphorylated peptides within the tyrosine kinase insert and carboxyl-terminal sequences of activated receptors
5 are commonly recognized by kinase substrates involved in signal transduction for the readjustment of gene expression in responding cells. Several families of receptor tyrosine kinases have been characterized. Van der Geer, *et al.*, *Ann. Rev. Cell Biol.*, 10:251-337 (1994). The major growth factors and receptors transducing angiogenic stimuli are schematically shown in Figure 1.

10 Fibroblast growth factors are also known to be involved in the regulation of angiogenesis. They have been shown to be mitogenic and chemotactic for cultured endothelial cells. Fibroblast growth factors also stimulate the production of proteases, such as collagenases and plasminogen activators, and induce tube formation by endothelial cells. Saksela, *et al.*,
15 *Ann. Rev. Cell Biol.*, 4:93-126 (1988). There are two general classes of fibroblast growth factors, FGF-1 and FGF-2, both of which lack conventional signal peptides. Both types have an affinity for heparin and FGF-2 is bound to heparin sulfate proteoglycans in the subendothelial extracellular matrix from which it may be released after injury. Heparin potentiates the stimulation of
20 endothelial cell proliferation by angiogenic FGFs, both by protecting against denaturation and degradation and dimerizing the FGFs. Cultured endothelial cells express the FGF-1 receptor but no significant levels of other high-affinity fibroblast growth factor receptors.

Among other ligands for receptor tyrosine kinases, the platelet
25 derived growth factor, PDGF-BB, has been shown to be weakly angiogenic in the chick chorioallantoic membrane. Risau, *et al.*, *Growth Factors*, 7:261-266 (1992). Transforming growth factor α (TGF α) is an angiogenic factor secreted by several tumor cell types and by macrophages. Hepatocyte growth factor (HGF), the ligand of the *c-met* proto-oncogene-encoded receptor, also is
30 strongly angiogenic.

Recent evidence shows that there are endothelial cell specific growth factors and receptors that may be primarily responsible for the stimulation of endothelial cell growth, differentiation and certain differentiated functions. The best studied of these is vascular endothelial growth factor
35 (VEGF), a member of the PDGF family. Vascular endothelial growth factor



- 1 -

RECEPTOR LIGAND

This is a continuation-in-part of United States Patent Application
Serial Number 08/510,133, filed August 1, 1995, ~~DI~~

FIELD OF THE INVENTION

5 The present invention generally relates to the field of genetic engineering and more particularly to growth factors for endothelial cells and growth factor genes.

BACKGROUND OF THE INVENTION

10 Developmental growth, the remodelling and regeneration of adult tissues, as well as solid tumor growth, can only occur when accompanied by blood vessel formation. Angioblasts and hematopoietic precursor cells differentiate from the mesoderm and form the blood islands of the yolk sac and the primary vascular system of the embryo. The development of blood vessels from these early (*in situ*) differentiating endothelial cells is termed
15 vasculogenesis. Major embryonic blood vessels are believed to arise via vasculogenesis, whereas the formation of the rest of the vascular tree is thought to occur as a result of vascular sprouting from pre-existing vessels, a process called angiogenesis, Risau, *et al.*, *Devel. Biol.*, 125:441-450 (1988).

20 Endothelial cells give rise to several types of functionally and morphologically distinct vessels. When organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases. Upon angiogenic stimulation, endothelial cells may re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their
25 tissue environment. Endothelial cells undergoing angiogenesis degrade the underlying basement membrane and migrate, forming capillary sprouts that project into the perivascular stroma. Ausprunk, *et al.*, *Microvasc. Rev.*, 14:51-65 (1977). Angiogenesis during tissue development and regeneration depends on the tightly controlled processes of endothelial cell proliferation,
30 migration, differentiation, and survival. Dysfunction of the endothelial cell regulatory system is a key feature of many diseases. Most significantly, tumor growth and metastasis have been shown to be angiogenesis dependent. Folkman, *et al.*, *J. Biol. Chem.*, 267:10931-10934 (1992).

08/585 895



- 52 -

ABSTRACT

Provided are ligands for the receptor tyrosine kinase, Flt4. Also provided are cDNAs and vectors encoding the ligand, pharmaceutical compositions and diagnostic reagents.

JOINT INVENTORS

"EXPRESS MAIL" mailing label No.
EG473137204US.

Date of Deposit: January 12, 1996

I hereby certify that this paper (or fee) is being
deposited with the United States Postal Service
"EXPRESS MAIL POST OFFICE TO ADDRESSEE"
service under 37 CFR \$1.10 on the date
indicated above and is addressed to: Assistant
Commissioner for Patents, Washington, D.C.
20231


David A. Gass

**APPLICATION FOR
UNITED STATES LETTERS PATENT**

SPECIFICATION

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Kari Alitalo, a citizen of Finland, residing at
Nyyrikintie 4A, 02100 Espoo, Finland, and Vladimir Joukov, a citizen of Finland,
residing at Topeliuksenkatu 32G8, 00290 Helsinki, Finland, have invented a
new and useful "RECEPTOR LIGAND", of which the following is a specification.

7. Deposit Account and Refund Authorization

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 or under other applicable rules (except payment of issue fees), to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.


Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Please direct all future communications to David A. Gass at the address below.

Respectfully submitted,

**MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN.**
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:


David A. Gass
Reg. No: 38,153

January 12, 1996

5. Filing Fee Calculation (37 CFR 1.16)

A. ☒ Utility Application

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	NO. FILED	NO. EXTRA	RATE	FEE	RATE	FEE
BASIC FEE				\$375.00		\$750.00
TOTAL	16 - 20	= 0	X 11 =	\$	X 22 =	\$
INDEP.	3 - 3	= 0	X 39 =	\$	X 78 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 125 =	\$	+ 250 =	
Filing Fee:				\$	OR	\$750.00

B. ☐ Design Application (\$150.00/\$300.00)

Filing Fee: \$ _____

C. ☐ Plant Application (\$245.00/\$490.00)

Filing Fee: \$ _____

D. Other Fees

☐ Recording Assignment [Fee -- \$40.00 per assignment] \$ _____

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached [Fee -- \$130.00] \$ _____

☐ Other \$ _____

Total Fees Enclosed **\$750.00**

6. Method of Payment of Fees

☒ Check in the amount of: **\$750.00**

☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

☐ Not enclosed

3. Declaration or Oath

- ☐ Enclosed
- ☐ Executed by (check all applicable boxes)
- ☐ Inventor(s)
- ☐ Legal representative of inventor(s)
(37 CFR 1.42 or 1.43)
- ☐ Joint inventor or person showing a proprietary interest on behalf of
inventor who refused to sign or cannot be reached
- ☐ The petition required by 37 CFR 1.47 and the statement required
by 37 CFR 1.47 are enclosed. See Item 5D below for fee.
- ☒ Not enclosed - the undersigned attorney or agent is authorized to file this
application on behalf of the applicant(s). An executed declaration will follow.

4. Additional Papers Enclosed

- ☐ Preliminary Amendment
- ☐ Information Disclosure Statement
- ☐ Declaration of Biological Deposit
- ☒ Computer-readable copy of sequence listing containing nucleotide and/or amino
acid sequence
- ☒ Statement pursuant to 37 C.F.R. §1.821(f)
- ☐ Verified statement(s) claiming small entity status under 37 CFR 1.9 and 1.27
- ☐ Associate Power of Attorney
- ☐ Verified translation of a non-English patent application
- ☐ An assignment of the invention
- ☐ Certified copy(ies) of application(s):

COUNTRY	APPLICATION NO.	FILED

from which priority under 35 USC 119 is claimed ☐ is(are) attached.

☐ will follow.

☐ Other



08 585

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No: 28113/33072

PATENT APPLICATION TRANSMITTAL

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Kari Alitalo and Vladimir Joukov

Title: "Receptor Ligand"

1. Type of Application

This new application is for a

- ☒ utility patent.
☐ design patent.

2. Application Papers Enclosed

- 1 Title Page
49 Pages of Specification (excluding Claims, Abstract & Drawings)
2 Pages of Claims
1 Page of Abstract
24 Sheets of Drawings (Figs. 1 to 18)
☐ Formal
☒ Informal

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on January 12, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EG473137204US.


David A. Gass

PATENT APPLICATION SERIAL NO. ~~00~~ 585895

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET



4130 105

0250 teams

#2 1/2

PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:) I hereby certify that this paper is being deposited
Alitalo et al.) with the United States Postal Service as first
Serial No.: 08/585,895) class mail, postage prepaid, in an envelope
Filed: January 12, 1996) addressed to: Assistant Commissioner for
For: Receptor Ligand) Patents, Washington, D.C. 20231, on this date:
Group Art Unit: Not yet assigned) Dated: March 28, 1996
Examiner: Not yet assigned) David A. Gass
David A. Gass
Registration No. 38,153
Attorney for Applicant(s)

TRANSMITTAL OF EXECUTED DECLARATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Attention: Application Branch

Sir:

Submitted herewith is an executed Declaration for filing in the above-identified application. No Notice to File Missing Parts has been received by the Applicants.

Also enclosed is a check in the amount of \$130.00 in payment of the fee for submission of the declaration. See 37 C.F.R. §1.16(e).

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this request is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

March 28, 1996

By: David A. Gass

David A. Gass
Reg. No: 38,153



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:)	I hereby certify that this paper is being deposited
Alitalo et al.)	with the United States Postal Service as first
Serial No.: 08/585,895)	class mail, postage prepaid, in an envelope
Filed: January 12, 1996)	addressed to: Assistant Commissioner for
For: Receptor Ligand)	Patents, Washington, D.C. 20231, on this date:
Group Art Unit: Not yet assigned)	Dated: <u>March 28, 1996</u>
Examiner: Not yet assigned)	<u>David A. Gass</u>
)	David A. Gass
)	Registration No. 38,153
)	Attorney for Applicant(s)

TRANSMITTAL OF EXECUTED DECLARATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Attention: Application Branch

Sir:

Submitted herewith is an executed Declaration for filing in the above-identified application. No Notice to File Missing Parts has been received by the Applicants.

Also enclosed is a check in the amount of \$130.00 in payment of the fee for submission of the declaration. See 37 C.F.R. §1.16(e).

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this request is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

March 28, 1996

By:

David A. Gass
David A. Gass
Reg. No: 38,153

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed on January 12, 1996, as Application Serial No. 08/585,895. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Priority Claimed
☐ Yes ☐ No

(Application Serial Number)	(Country)	(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

(Application Serial Number)	(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)
08/510,133	01 August 1995	Pending

(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shulman (19,412)	Trevor B. Joike (25,542)	Richard A. Schurr (30,890)	James J. Napoli (32,361)
Donald J. Broff (19,420)	Timothy J. Vezau (26,348)	Anthony Nimmo (30,920)	Richard M. La Barge (32,254)
Owen J. Murray (22,111)	Carl E. Moore, Jr. (26,487)	Christine A. Dudzik (31,245)	Jeffrey W. Smith (33,455)
Allen H. Gerstein (22,218)	Richard H. Anderson (26,526)	Kevin D. Hogg (31,839)	Douglas C. Hochstetler (33,710)
Nate F. Scarpelli (22,320)	Patrick D. Ertel (26,877)	Jeffrey S. Sharp (31,879)	Cynthia L. Schaller (34,245)
Edward M. O'Toole (22,477)	James P. Zeller (28,491)	Donald J. Pochopien (32,167)	Robert M. Gerstein (34,824)
Michael F. Borun (25,447)	William E. McCracken (30,195)	Martin J. Hirsch (32,237)	David A. Gass (38,153)

Send correspondence to: David A. Gass

FIRM NAME	PHONE NO.	STREET	CITY & STATE	ZIP CODE
Marshall, O'Toole, Gerstein, Murray & Borun	312-474-6300	6300 Sears Tower 233 South Wacker Drive	Chicago, Illinois	60606-6402

Full Name of First or Joint Inventor	Kari Alitalo	Country	Finland
Residence Address - Street	Nyyrikintie 4A	Post Office Address - Street	Same
City (Zip)	02100 Espoo	City (Zip)	Same
State or Country	FINLAND	State or Country	Same
Date	March 14, 1996	Signature	[Signature]

See second page for additional inventor

See reverse for relevant rules & statutes

Second Joint Inventor, if any Vladimir Joukov	Citizenship Finland <i>Russia</i>
Residence Address - Street Topeliuksenkatu 32G8	Post Office Address - Street Same
City (Zip) 00290 Helsinki <i>FI</i>	City (Zip) Same
State or Country FINLAND	State or Country Same
Date <input checked="" type="checkbox"/> <i>March 14, 1996</i>	Signature <input checked="" type="checkbox"/> <i>V. Joukov</i>



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
08/385,295	01/11/96	DAVID A. SASS	08113/00072

0272/0913
DAVID A. SASS
MARSHALL C. TOOLE GERSTEIN MURRAY J. BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

DATE MAILED: 05/15/96

NOTICE TO FILE MISSING PARTS OF APPLICATION FILING DATE GRANTED

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 150.00 for large entities or \$ 15.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 150.00.

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit \$ _____ to complete the basic filing fee.
2. ☐ Additional claim fees of \$ _____ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
3. ☒ The oath or declaration:
☒ is missing.
☐ does not cover the newly submitted items.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.
4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
5. ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:
_____. An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ _____ under 37 CFR 1.17(k), unless this fee has already been paid.
8. ☐ A \$ _____ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).
9. ☐ Your filing receipt was mailed in error because your check was returned without payment.
10. ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with Sequence Rules 37 CFR 1.821-1.825.
11. ☐ Other.

Direct the response to Box Missing Part and refer any questions to the Customer Service Center at (703) 308-1202.

A copy of this notice MUST be returned with the response.





#130

122

0300
5/16/96PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.

Serial No: 08/585,895

Filed: January 16, 1996

Title: RECEPTOR LIGAND

Group Art Unit: Not yet assigned

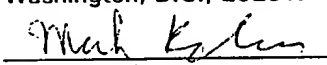
Examiner: Not yet assigned

"EXPRESS MAIL"

Mailing label No. EM118660766US

Date of Deposit:
May 24, 1996

I hereby certify that this paper (or fee)
is being deposited with the United
States Postal Service "EXPRESS
MAIL POST OFFICE TO ADDRESSEE"
service under 37 CFR §1.10 on the
date indicated above and is addressed
to the Assistant Commissioner for
Patents,
Washington, D.C., 20231.


Mark Bonadonna

Petition to Accord a Filing Date of January 12, 1996,
Pursuant to 37 C.F.R. §§ 1.6, 1.10,
and 1.53, and M.P.E.P. §506.02

or, in the alternative,

Petition to Suspend Rules Pursuant to
35 U.S.C. §21 and 37 C.F.R. § 1.183
to Accord Filing Date of January 12, 1996

Assistant Commissioner for Patents
Washington, D.C. 20231

Attn: Special Program Law Office

Dear Sir:

The Applicants request that the above-identified patent application,
which has been accorded a filing date of January 16, 1996, be accorded an earlier
filing date of January 12, 1996. The application was filed in accordance with 37
C.F.R. §1.10 and 1.53(a) on January 12, 1996.

If this request is denied, then the Applicants hereby petition to accord
a filing date of January 12, 1996, pursuant to 37 C.F.R. §§ 1.6, 1.10, and 1.53,
and M.P.E.P. §506.02. In the alternative, the Applicants petition to suspend the
rules pursuant to 35 U.S.C. §21 and 37 C.F.R. §1.183, to Accord Filing Date of
January 12, 1996.

I. **Petition for Review of Refusal to Accord Filing Date Pursuant to 37 C.F.R. §§ 1.6, 1.10, 1.17(h) and 1.53, and M.P.E.P. §506.02**

A. **Statement of Facts**

The above-identified patent application was filed in accordance with 37 C.F.R. §1.53(a) on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10. Copies of the Applicants' transmittal letter, specification cover sheet, and express mail mailing receipt are submitted herewith as Exhibits 1, 2, and 3, respectively. The transmittal letter and specification cover sheet contain certificates of mailing in accordance with 37 C.F.R. §1.10, dated January 12, 1996.

On May 15, 1996, the Patent and Trademark Office mailed a Notice to File Missing Parts of Application -- Filing Date Granted. (Exhibit 4.) However, the filing date on the Notice was Tuesday, January 16, 1996, instead of January 12, 1996.

B. **Argument**

The Patent and Trademark Office has not identified any defects in the application or certificates of mailing dated Friday, January 12, 1996. Friday, January 12, 1996, was not a Saturday, Sunday, or Federal holiday.¹ Accordingly, under the rules promulgated by the Commissioner, the above-identified application properly should be considered as having been filed on January 12, 1996. See 37 C.F.R. §1.10 (a). Correction of the filing date to January 12, 1996, is respectfully requested.

II. **Conditional Petition to Suspend Rules to Accord a Filing Date of January 12, 1996**

If the foregoing petition is denied on the grounds that the Commissioner declared January 12, 1996, to be a "Federal holiday" as that term is used in 37 C.F.R. §1.10, then the Applicants hereby petition the Commissioner to suspend the rules pursuant to 37 C.F.R. §1.183, and to accord the present application a filing date of January 12, 1996. This petition has been filed following a telephone interview between the undersigned attorney and Examiner

¹ As set forth in M.P.E.P. §710.05, the Federal holidays are New Year Day, Martin Luther King's Birthday, Washington's Birthday, Memorial Day, Independence Day, Labor Day, Columbus Day, Veteran's Day, Thanksgiving Day, Christmas Day, and Inauguration Day.

Nguyen concerning this matter on May 20, 1996, which interview the Applicants acknowledge with thanks.

A. Statement of Facts

The Applicants, through their attorneys at Marshall, O'Toole, Gerstein, Murray & Borun, prepared the above-identified application for filing on or before January 12, 1996, on the informed belief that a manuscript (authored by the Applicants and others) describing aspects of the invention would be published in *The EMBO Journal*, Volume 15, Number 2, on January 15, 1996 (Martin Luther King's Birthday, a Federal Holiday). The Application was filed on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10; as explained in detail in Section I above. (See Exhibits 1-3.) However, the application was accorded a filing date of January 16, 1996. (See Exhibit 4.)

The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

B. Argument

The Applicants' reliance on the Express Mail procedures of 37 C.F.R. §1.10 for the present application, to obtain a filing date of January 12, 1996, was reasonable, because January 12, 1996, was not a Saturday, Sunday, or recognized Federal holiday. If January 12 was deemed a "Federal holiday" by the Commissioner, it was so deemed, without advance warning to the Applicants, due to an unscheduled and unforeseeable event -- adverse weather conditions in the District of Columbia. An apparent purpose of deeming such days to be "Federal holidays" is to protect applicants' patent rights, by allowing for the timely filing of papers or fees on the next succeeding business day. See M.P.E.P. §510. However, the effect of denying the present Applicants a filing date of January 12, 1996, on the grounds of an unscheduled, weather-related "Federal holiday" being declared, may be to destroy the present Applicants' valuable patent rights in foreign countries. The Applicants submit that the foregoing unforeseeable circumstances comprise an extraordinary situation, and that justice requires the suspension of rules to accord the present application a filing date of January 12,


1996, to preserve the Applicants' foreign patent rights. The granting of such a filing date is not believed to contravene any requirements of the patent statutes. In fact, the granting of the January 12, 1996, filing date is submitted to be in complete harmony with the purpose and intent of 35 U.S.C. §21 and 37 C.F.R. §§1.6 and 1.10.

SUMMARY

The Applicants respectfully request and petition that the present application be accorded a filing date of January 12, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

Dated: MAY 24, 1996

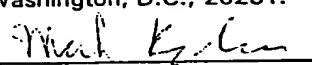

David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.)	"EXPRESS MAIL"
)	Mailing label No. EM118660766US
Serial No: 08/585,895)	
Filed: January 16, 1996)	Date of Deposit:
)	May 24, 1996
Title: RECEPTOR LIGAND)	
Group Art Unit: Not yet assigned)	I hereby certify that this paper (or fee)
Examiner: Not yet assigned)	is being deposited with the United
)	States Postal Service "EXPRESS
)	MAIL POST OFFICE TO ADDRESSEE"
)	service under 37 CFR §1.10 on the
)	date indicated above and is addressed
)	to the Assistant Commissioner for
)	Patents,
)	Washington, D.C., 20231.
)	
)	Mark Bonadonna

Petition to Accord a Filing Date of January 12, 1996,
Pursuant to 37 C.F.R. §§ 1.6, 1.10,
and 1.53, and M.P.E.P. §506.02

or, in the alternative,

Petition to Suspend Rules Pursuant to
35 U.S.C. §21 and 37 C.F.R. § 1.183
to Accord Filing Date of January 12, 1996

Assistant Commissioner for Patents
Washington, D.C. 20231

Attn: Special Program Law Office

Dear Sir:

The Applicants request that the above-identified patent application, which has been accorded a filing date of January 16, 1996, be accorded an earlier filing date of January 12, 1996. The application was filed in accordance with 37 C.F.R. §1.10 and 1.53(a) on January 12, 1996.

If this request is denied, then the Applicants hereby petition to accord a filing date of January 12, 1996, pursuant to 37 C.F.R. §§ 1.6, 1.10, and 1.53, and M.P.E.P. §506.02. In the alternative, the Applicants petition to suspend the rules pursuant to 35 U.S.C. §21 and 37 C.F.R. §1.183, to Accord Filing Date of January 12, 1996.

I. Petition for Review of Refusal to Accord Filing Date Pursuant to 37 C.F.R. §§ 1.6, 1.10, 1.17(h) and 1.53, and M.P.E.P. §506.02

A. Statement of Facts

The above-identified patent application was filed in accordance with 37 C.F.R. §1.53(a) on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10. Copies of the Applicants' transmittal letter, specification cover sheet, and express mail mailing receipt are submitted herewith as Exhibits 1, 2, and 3, respectively. The transmittal letter and specification cover sheet contain certificates of mailing in accordance with 37 C.F.R. §1.10, dated January 12, 1996.

On May 15, 1996, the Patent and Trademark Office mailed a Notice to File Missing Parts of Application -- Filing Date Granted. (Exhibit 4.) However, the filing date on the Notice was Tuesday, January 16, 1996, instead of January 12, 1996.

B. Argument

The Patent and Trademark Office has not identified any defects in the application or certificates of mailing dated Friday, January 12, 1996. Friday, January 12, 1996, was not a Saturday, Sunday, or Federal holiday.¹ Accordingly, under the rules promulgated by the Commissioner, the above-identified application properly should be considered as having been filed on January 12, 1996. See 37 C.F.R. §1.10 (a). Correction of the filing date to January 12, 1996, is respectfully requested.

II. Conditional Petition to Suspend Rules to Accord a Filing Date of January 12, 1996

If the foregoing petition is denied on the grounds that the Commissioner declared January 12, 1996, to be a "Federal holiday" as that term is used in 37 C.F.R. §1.10, then the Applicants hereby petition the Commissioner to suspend the rules pursuant to 37 C.F.R. §1.183, and to accord the present application a filing date of January 12, 1996. This petition has been filed following a telephone interview between the undersigned attorney and Examiner

¹ As set forth in M.P.E.P. §710.05, the Federal holidays are New Year Day, Martin Luther King's Birthday, Washington's Birthday, Memorial Day, Independence Day, Labor Day, Columbus Day, Veteran's Day, Thanksgiving Day, Christmas Day, and Inauguration Day.

Nguyen concerning this matter on May 20, 1996, which interview the Applicants acknowledge with thanks.

A. Statement of Facts

The Applicants, through their attorneys at Marshall, O'Toole, Gerstein, Murray & Borun, prepared the above-identified application for filing on or before January 12, 1996, on the informed belief that a manuscript (authored by the Applicants and others) describing aspects of the invention would be published in *The EMBO Journal*, Volume 15, Number 2, on January 15, 1996 (Martin Luther King's Birthday, a Federal Holiday). The Application was filed on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10, as explained in detail in Section I above. (See Exhibits 1-3.) However, the application was accorded a filing date of January 16, 1996. (See Exhibit 4.)

The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

B. Argument

The Applicants' reliance on the Express Mail procedures of 37 C.F.R. §1.10 for the present application, to obtain a filing date of January 12, 1996, was reasonable, because January 12, 1996, was not a Saturday, Sunday, or recognized Federal holiday. If January 12 was deemed a "Federal holiday" by the Commissioner, it was so deemed, without advance warning to the Applicants, due to an unscheduled and unforeseeable event -- adverse weather conditions in the District of Columbia. An apparent purpose of deeming such days to be "Federal holidays" is to protect applicants' patent rights, by allowing for the timely filing of papers or fees on the next succeeding business day. See M.P.E.P. §510. However, the effect of denying the present Applicants a filing date of January 12, 1996, on the grounds of an unscheduled, weather-related "Federal holiday" being declared, may be to destroy the present Applicants' valuable patent rights in foreign countries. The Applicants submit that the foregoing unforeseeable circumstances comprise an extraordinary situation, and that justice requires the suspension of rules to accord the present application a filing date of January 12,

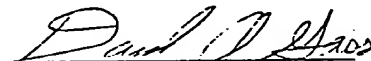
1996, to preserve the Applicants' foreign patent rights. The granting of such a filing date is not believed to contravene any requirements of the patent statutes. In fact, the granting of the January 12, 1996, filing date is submitted to be in complete harmony with the purpose and intent of 35 U.S.C. §21 and 37 C.F.R. §§1.6 and 1.10.

SUMMARY

The Applicants respectfully request and petition that the present application be accorded a filing date of January 12, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

Dated: MAY 24, 1996



David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300



Exhibit 1

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No: 28113/33072

PATENT APPLICATION TRANSMITTAL

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Kari Alitalo and Vladimir Joukov

Title: "Receptor Ligand"

1. Type of Application

This new application is for a

- ☒ utility patent.
☐ design patent.

2. Application Papers Enclosed

- | | |
|-------------------------------------|--|
| 1 | Title Page |
| 49 | Pages of Specification (excluding Claims, Abstract & Drawings) |
| 2 | Pages of Claims |
| 1 | Page of Abstract |
| 24 | Sheets of Drawings (Figs. 1 to 18) |
| <input type="checkbox"/> | Formal |
| <input checked="" type="checkbox"/> | Informal |

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on January 12, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EG473137204US.


David A. Gass

3. Declaration or Oath

☐ Enclosed

☐ Executed by (check all applicable boxes)

☐ Inventor(s)

☐ Legal representative of inventor(s)
(37 CFR 1.42 or 1.43)

☐ Joint inventor or person showing a proprietary interest on behalf of
inventor who refused to sign or cannot be reached

☐ The petition required by 37 CFR 1.47 and the statement required
by 37 CFR 1.47 are enclosed. See Item 5D below for fee.

☒ Not enclosed - the undersigned attorney or agent is authorized to file this
application on behalf of the applicant(s). An executed declaration will follow.

4. Additional Papers Enclosed

☐ Preliminary Amendment

☐ Information Disclosure Statement

☐ Declaration of Biological Deposit

☒ Computer-readable copy of sequence listing containing nucleotide and/or amino
acid sequence

☒ Statement pursuant to 37 C.F.R. §1.821(f)

☐ Verified statement(s) claiming small entity status under 37 CFR 1.9 and 1.27

☐ Associate Power of Attorney

☐ Verified translation of a non-English patent application

☐ An assignment of the invention

☐ Certified copy(ies) of application(s):

COUNTRY	APPLICATION NO.	FILED

from which priority under 35 USC 119 is claimed ☐ is(are) attached.

☐ will follow.

☐ Other

5. Filing Fee Calculation (37 CFR 1.16)

A. ☒ Utility Application

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	NO. FILED	NO. EXTRA	RATE	FEE	RATE	FEE
BASIC FEE				\$375.00		\$750.00
TOTAL	16 - 20	= 0	X 11 =	\$	X 22 =	\$
INDEP.	3 - 3	= 0	X 39 =	\$	X 78 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 125 =	\$	+ 250 =	
Filing Fee:				\$	OR	\$750.00

B. ☐ Design Application (\$150.00/\$300.00)

Filing Fee: \$ _____

C. ☐ Plant Application (\$245.00/\$490.00)

Filing Fee: \$ _____

D. Other Fees

☐ Recording Assignment [Fee -- \$40.00 per assignment] \$ _____

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached [Fee -- \$130.00] \$ _____

☐ Other \$ _____

Total Fees Enclosed \$750.00

6. Method of Payment of Fees

☒ Check in the amount of: \$750.00

☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

☐ Not enclosed

7. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 or under other applicable rules (except payment of issue fees), to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Please direct all future communications to David A. Gass at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:


David A. Gass
Reg. No: 38,153

January 12, 1996



JOINT INVENTORS

Exhibit 2

"EXPRESS MAIL" mailing label No.
EG473137204US.

Date of Deposit: January 12, 1996

I hereby certify that this paper (or fee) is being
deposited with the United States Postal Service
"EXPRESS MAIL POST OFFICE TO ADDRESSEE"
service under 37 CFR §1.10 on the date
indicated above and is addressed to: Assistant
Commissioner for Patents, Washington, D.C.
20231


David A. Gass

APPLICATION FOR
UNITED STATES LETTERS PATENT

SPECIFICATION

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Kari Alitalo, a citizen of Finland, residing at
Nyyrikintie 4A, 02100 Espoo, Finland, and Vladimir Joukov, a citizen of Finland,
residing at Topeliuksenkatu 32G8, 00290 Helsinki, Finland, have invented a
new and useful "RECEPTOR LIGAND", of which the following is a specification.



Exhibit 3

POST OFFICE TO ADDRESSEE		EXPRESS MAIL		EG473137204US	
ORIGIN (POSTAL USE ONLY)					
Postage		Day of Delivery		Red Rate Envelope	
No. 1296		12 Noon		15.00	
Time in AM		12 Noon		15.00	
Weight 1 lb 8 oz		Int'l Alpha Country Code		Total Postage & Fees	
No Delivery		Acceptance		15.00	
Weekend		Clerk Initials			
CUSTOMER USE ONLY					
METHOD OF PAYMENT:					
Express Mail Corporate Acct. No.					
Federal Agency Acct. No. or Postal Service Acct. No.					
FROM: (PLEASE PRINT)			TO: (PLEASE PRINT)		
28113/38072			Assistant Commissioner		
DAVID A. GASS			for Patents		
MARSHALL, O'TOOLE, GERSTEIN,			Washington, D.C. 20231		
MURRAY & BORUN					
6300 SEARS TOWER					
233 SOUTH WACKER DRIVE					
CHICAGO, ILLINOIS 60606-6402					
Box Patent Application					

DEL 11-8 11/93

For Pickup or Tracking Call 1-800-222-1811

CUSTOMER COPY



Exhibit 4
UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
08/585,895	01/16/96	ALITALO	K 28113/33072

0272/0515
DAVID A GASS
MARSHALL O'TOOLE GERSTEIN MURRAY & BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

RECEIVED
MAY 20 1996

6-15-96 MARSHALL O'TOOLE

DATE MAILED: 05/15/96

NOTICE TO FILE MISSING PARTS OF APPLICATION FILING DATE GRANTED

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 130.00 for large entities or \$ 65.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 130.00.

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit \$ _____ to complete the basic filing fee.
2. ☐ Additional claim fees of \$ _____ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
3. ☒ The oath or declaration:
☒ is missing.
☐ does not cover the newly submitted items.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.
4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
5. ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:

An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ _____ under 37 CFR 1.17(k), unless this fee has already been paid.
8. ☐ A \$ _____ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).
9. ☐ Your filing receipt was mailed in error because your check was returned without payment.
10. ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with Sequence Rules 37 CFR 1.821-1.825.
11. ☐ Other.

Direct the response to Box Missing Part and refer any questions to the Customer Service Center at (703) 308-1202.

A copy of this notice MUST be returned with the response.

ATTORNEY'S/APPLICANTS COPY





130-122 B

PATENT # K
28113/33072
NOTE PA
NUMBER
FIVE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.

Serial No: 08/585,895

Filed: January 16, 1996

Title: RECEPTOR LIGAND

Group Art Unit: Not yet assigned

Examiner: Not yet assigned

"EXPRESS MAIL"

Mailing label No.: EM118663762US

Date of Deposit:
June 25, 1996

I hereby certify that this paper (or fee)
is being deposited with the United
States Postal Service "EXPRESS
MAIL POST OFFICE TO ADDRESSEE"
service under 37 CFR §1.10 on the
date indicated above and is addressed
to the Assistant Commissioner for
Patents,
Washington, D.C., 20231.

Mark Bonadonna
Mark Bonadonna

Petition to Expedite Handling of Earlier Filed Petition
Pursuant to 37 C.F.R. §§ 1.181-183

Assistant Commissioner for Patents
Washington, D.C. 20231

Attn: Petitions Office

Dear Sir:

On May 24, 1996, the Applicants filed a petition (the "Filing Date Petition") requesting that the above-identified patent application, which has been accorded a filing date of January 16, 1996, be accorded an earlier filing date of January 12, 1996. For the reasons set forth below, the Applicants request that the Filing Date Petition receive expedited handling. This request is accompanied by a check for \$130 in payment of the petition fee.

The Applicants request expedited handling for the purposes of international filings which will claim priority from the present application.

A. Statement of Facts

The Applicants, through their attorneys at Marshall, O'Toole, Gerstein, Murray & Borun, prepared the above-identified application for filing on or before January 12, 1996, on the informed belief that a manuscript (authored by the Applicants and others) describing aspects of the invention would be published in *The EMBO Journal*, Volume 15, Number 2, on January 15, 1996 (Martin Luther King's Birthday, a Federal holiday). The Application was filed on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10. However, the application was accorded a filing date of the following Tuesday, January 16, 1996. On May 24, 1996, promptly after receiving a Notice to File Missing Parts dated May 15, 1996, the Applicants filed the Filing Date Petition, setting forth reasons and facts why the present application should be accorded a filing date of January 12, 1996.

The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

The present application is a continuation-in-part of U.S. Patent Application Serial No. 08/510,133, filed August 1, 1995. To obtain the priority benefit of both the parent application and the present application under the Paris Convention, the Applicants intend to file at least one foreign application on or before August 1, 1996.

B. Argument

The Applicants request an expedited decision on the Filing Date Petition to permit the Applicants to make an informed and accurate foreign filing that claims priority from the above-identified application. Specifically, the Applicants request that the Patent Office render its decision on the Filing Date Petition substantially in advance of the August 1, 1996.

First, the decision on the Filing Date Petition will impact the accuracy of the Applicants' foreign filings. It is important to identify priority documents in foreign filings with particularity, e.g., by serial number and filing date. A decision

on the Filing Date Petition in advance of the August 1, 1996, Paris Convention deadline will permit the Applicants to identify this priority application by its official filing date.

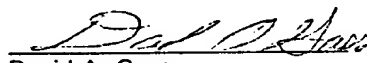
Second, a decision on the Filing Date Petition, substantially in advance of the August 1, 1996, treaty deadline, will permit the Applicants to evaluate and properly claim the subject matter of their invention in foreign applications. As explained in the Filing Date petition, the effect of denying the present Applicants a filing date of January 12, 1996, may be to destroy the present Applicants' valuable patent rights in foreign countries. A decision on the Filing Date Petition substantially in advance of the August 1, 1996, deadline will permit the Applicants to properly evaluate the value of the present application as a priority document under the patent laws of "absolute novelty" countries.

SUMMARY

The Applicants respectfully request and petition that their earlier-filed petition (to accord the present application a filing date of January 12, 1996) be handled expeditiously, such that a decision is rendered substantially in advance of August 1, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

Dated: 25 June 1996


David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.)	"EXPRESS MAIL"
Serial No: 08/585,895)	Mailing label No.: EM118663762US
Filed: January 16, 1996)	Date of Deposit:
Title: RECEPTOR LIGAND)	June 25, 1996
Group Art Unit: Not yet assigned)	I hereby certify that this paper (or fee)
Examiner: Not yet assigned)	is being deposited with the United
)	States Postal Service "EXPRESS
)	MAIL POST OFFICE TO ADDRESSEE"
)	service under 37 CFR §1.10 on the
)	date indicated above and is addressed
)	to the Assistant Commissioner for
)	Patents,
)	Washington, D.C., 20231.
)	<i>Mark Bonadonna</i>
)	Mark Bonadonna

Petition to Expedite Handling of Earlier Filed Petition
Pursuant to 37 C.F.R. §§ 1.181-183

Assistant Commissioner for Patents
Washington, D.C. 20231

Attn: Petitions Office

Dear Sir:

On May 24, 1996, the Applicants filed a petition (the "Filing Date Petition") requesting that the above-identified patent application, which has been accorded a filing date of January 16, 1996, be accorded an earlier filing date of January 12, 1996. For the reasons set forth below, the Applicants request that the Filing Date Petition receive expedited handling. This request is accompanied by a check for \$130 in payment of the petition fee.

The Applicants request expedited handling for the purposes of international filings which will claim priority from the present application.

A. Statement of Facts

The Applicants, through their attorneys at Marshall, O'Toole, Gerstein, Murray & Borun, prepared the above-identified application for filing on or before January 12, 1996, on the informed belief that a manuscript (authored by the Applicants and others) describing aspects of the invention would be published in *The EMBO Journal*, Volume 15, Number 2, on January 15, 1996 (Martin Luther King's Birthday, a Federal holiday). The Application was filed on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10. However, the application was accorded a filing date of the following Tuesday, January 16, 1996. On May 24, 1996, promptly after receiving a Notice to File Missing Parts dated May 15, 1996, the Applicants filed the Filing Date Petition, setting forth reasons and facts why the present application should be accorded a filing date of January 12, 1996.

The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

The present application is a continuation-in-part of U.S. Patent Application Serial No. 08/510,133, filed August 1, 1995. To obtain the priority benefit of both the parent application and the present application under the Paris Convention, the Applicants intend to file at least one foreign application on or before August 1, 1996.

B. Argument

The Applicants request an expedited decision on the Filing Date Petition to permit the Applicants to make an informed and accurate foreign filing that claims priority from the above-identified application. Specifically, the Applicants request that the Patent Office render its decision on the Filing Date Petition substantially in advance of the August 1, 1996.

First, the decision on the Filing Date Petition will impact the accuracy of the Applicants' foreign filings. It is important to identify priority documents in foreign filings with particularity, e.g., by serial number and filing date. A decision

on the Filing Date Petition in advance of the August 1, 1996, Paris Convention deadline will permit the Applicants to identify this priority application by its official filing date.

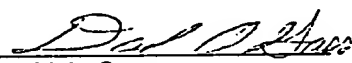
Second, a decision on the Filing Date Petition, substantially in advance of the August 1, 1996, treaty deadline, will permit the Applicants to evaluate and properly claim the subject matter of their invention in foreign applications. As explained in the Filing Date petition, the effect of denying the present Applicants a filing date of January 12, 1996, may be to destroy the present Applicants' valuable patent rights in foreign countries. A decision on the Filing Date Petition substantially in advance of the August 1, 1996, deadline will permit the Applicants to properly evaluate the value of the present application as a priority document under the patent laws of "absolute novelty" countries.

SUMMARY

The Applicants respectfully request and petition that their earlier-filed petition (to accord the present application a filing date of January 12, 1996) be handled expeditiously, such that a decision is rendered substantially in advance of August 1, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

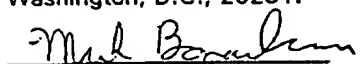
Dated: 25 June 1996


David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.)	"EXPRESS MAIL"
)	Mailing label No.: EM118663762US
Serial No: 08/585,895)	
)	Date of Deposit:
Filed: January 16, 1996)	June 25, 1996
)	
Title: RECEPTOR LIGAND)	I hereby certify that this paper (or fee)
)	is being deposited with the United
Group Art Unit: Not yet assigned)	States Postal Service "EXPRESS
)	MAIL POST OFFICE TO ADDRESSEE"
Examiner: Not yet assigned)	service under 37 CFR §1.10 on the
)	date indicated above and is addressed
)	to the Assistant Commissioner for
)	Patents,
)	Washington, D.C., 20231.
)	
)	Mark Bonadonna

Petition to Expedite Handling of Earlier Filed Petition
Pursuant to 37 C.F.R. §§ 1.181-183

Assistant Commissioner for Patents
Washington, D.C. 20231

Attn: Petitions Office

Dear Sir:

On May 24, 1996, the Applicants filed a petition (the "Filing Date Petition") requesting that the above-identified patent application, which has been accorded a filing date of January 16, 1996, be accorded an earlier filing date of January 12, 1996. For the reasons set forth below, the Applicants request that the Filing Date Petition receive expedited handling. This request is accompanied by a check for \$130 in payment of the petition fee.

FAX RECEIVED

JUL 17 1996

PETITION OFFICE

The Applicants request expedited handling for the purposes of international filings which will claim priority from the present application.

A. Statement of Facts

The Applicants, through their attorneys at Marshall, O'Toole, Gerstein, Murray & Borun, prepared the above-identified application for filing on or before January 12, 1996, on the informed belief that a manuscript (authored by the Applicants and others) describing aspects of the invention would be published in *The EMBO Journal*, Volume 15, Number 2, on January 15, 1996 (Martin Luther King's Birthday, a Federal holiday). The Application was filed on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10. However, the application was accorded a filing date of the following Tuesday, January 16, 1996. On May 24, 1996, promptly after receiving a Notice to File Missing Parts dated May 15, 1996, the Applicants filed the Filing Date Petition, setting forth reasons and facts why the present application should be accorded a filing date of January 12, 1996.

The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

The present application is a continuation-in-part of U.S. Patent Application Serial No. 08/510,133, filed August 1, 1995. To obtain the priority benefit of both the parent application and the present application under the Paris Convention, the Applicants intend to file at least one foreign application on or before August 1, 1996.

B. Argument

The Applicants request an expedited decision on the Filing Date Petition to permit the Applicants to make an informed and accurate foreign filing that claims priority from the above-identified application. Specifically, the Applicants request that the Patent Office render its decision on the Filing Date Petition substantially in advance of the August 1, 1996.

First, the decision on the Filing Date Petition will impact the accuracy of the Applicants' foreign filings. It is important to identify priority documents in foreign filings with particularity, e.g., by serial number and filing date. A decision

on the Filing Date Petition in advance of the August 1, 1996, Paris Convention deadline will permit the Applicants to identify this priority application by its official filing date.

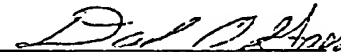
Second, a decision on the Filing Date Petition, substantially in advance of the August 1, 1996, treaty deadline, will permit the Applicants to evaluate and properly claim the subject matter of their invention in foreign applications. As explained in the Filing Date petition, the effect of denying the present Applicants a filing date of January 12, 1996, may be to destroy the present Applicants' valuable patent rights in foreign countries. A decision on the Filing Date Petition substantially in advance of the August 1, 1996, deadline will permit the Applicants to properly evaluate the value of the present application as a priority document under the patent laws of "absolute novelty" countries.

SUMMARY

The Applicants respectfully request and petition that their earlier-filed petition (to accord the present application a filing date of January 12, 1996) be handled expeditiously, such that a decision is rendered substantially in advance of August 1, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

Dated: 25 June 1996


David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

*** ACTIVITY REPORT ***

RECEPTION OK

TX/RX NO. 9328

CONNECTION TEL 3124740448

CONNECTION ID

START TIME 07/17 10:57

USAGE TIME 01'16

PAGES 4

RESULT OK

Jul. 17, 1996 9:54AM MARSHALL, O'TOOLE

No. 1196 P. 1/4
From: 0808

MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BORUN

RICHARD H. ANDERSON
MICHAEL F. BORUN
DONALD J. BROTT
MADELINE MENRICKS DEVEREUX
CHRISTINE A. DUDZIK
PATRICK D. EITEL
ALLEN H. GERSTEIN
ROBERT M. GERSTEIN
MARTIN J. HIRSCH
DOUGLASS C. HOCHSTETLER
KEVIN D. HOGG
TREVOR B. JOIKE
RICHARD M. LA BARGE
WILLIAM E. McCracken
CARL E. MOORE, JR.
OWEN J. MURRAY
JAMES J. NAPOLI, Ph.D.
ANTHONY NIMMO
EDWARD M. O'TOOLE
DONALD J. POCHOPIEN, Ph.D.
NATE F. SCARPELLO
CYNTHIA L. SCHALLER
RICHARD A. SCHINURR
JEFFREY S. SHARP
ALVIN D. SHULMAN
JEFFREY W. SMITH
TIMOTHY J. VEZEAU
KARL A. VICK
JAMES P. ZELLER

ATTORNEYS AT LAW
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606-6402
(312) 474-6300
FAX: (312) 474-0448

G. CHRISTOPHER BRAIDWOOD
RICHARD A. BRANDON
JANE J. CHOI
DAVID W. CLOUGH, Ph.D.
DAVID A. GASS
SCOTT M. GETTLESON
MICHAEL R. GRAHAM
ROGER A. HEPPERMANN
DANIELLE M. JOHNSTON
GREGORY C. MAYER
WILLIAM K. MERKEL, Ph.D.
STEPHEN M. MILLER
LI-HSIEN RIN-LAUREN, M.D.
DOUGLAS H. SIEGEL
YOUNG J. SUH, Ph.D.

OF COUNSEL
JOHN H. COULT

REGISTERED PATENT AGENTS
GRETA E. NOLAND
JOSEPH A. WILLIAMS, JR., Ph.D.

July 17, 1996

FACSIMILE
TRANSMISSION SHEET

TO: Tim Heightbrink
FAX NO.: (703) 308-6916

CLIENT NO.: 28113

MATTER NO.: 33072

FROM: David A. Gass

COUNTRY CODE: US

FAX NO.: (312) 474-0448

PAGES (INCLUDING THIS PAGE): 4

PLEASE CONFIRM RECEIPT: _____

MESSAGE: _____

FAX RECEIVED

JUL 17 1996

PATENT OFFICE

Please contact _____ at 474-6300 if you do
not receive all of the pages in good condition.

The material of this transmission contains confidential information intended only for the addressee. If you are not the addressee, any disclosure or use of this information by you is strictly prohibited. If you have received this facsimile in error, please notify us by telephone immediately.





UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Paper No. 7

David A. Gass
Marshall, O'Toole, Gerstein,
Murray & Borun
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402

COPY MAILED

JUL 22 1996

**OFFICE OF PETITIONS
AND TRADEMARKS**

In re Application of
Alitalo et al.
Application No. 08/585,895
Filed: January 12, 1996
Attorney Docket No. 28113/33072

DECISION GRANTING PETITION

This is a decision on the petition filed May 24, 1996 made special by the petition filed by "Express Mail" June 25, 1996 (copy of the July 25, 1996 petition sent July 17, 1996, by facsimile transmission in response to a telephone communication by Tim Heitbrink, of the Office of Petitions), requesting that the above-identified application be accorded a filing date of January 12, 1996.

The application, which is a continuation-in-part application under 37 CFR 1.53, was deposited in Express Mail service on January 12, 1996, which was a Friday. The Express Mail label number was placed on the papers. However, Federal and District of Columbia government offices, including the Patent and Trademark Office (Office), were officially closed for the entire day on January 12, 1996, as a result of adverse weather conditions. Under such conditions, the Office considers that day as a "federal holiday within the District of Columbia" under 35 U.S.C. 21. See 1183 OG 60 and notice entitled "Filing Of Papers During Unscheduled Closings Of The Patent and Trademark Office", originally published at 1097 OG 53, reprinted at 1158 OG 8 (copies enclosed).

In accordance with Office procedure, the present application was accorded a filing date of Tuesday, January 16, 1996, the next business day following the date of deposit in Express Mail service. The fact that no papers are received or stamped on Saturdays, Sundays or Federal holidays within the District of Columbia and the handling of Express Mail in such cases is clearly set out in 37 CFR 1.6(a) and 1.10(a).

Of course, "in an extraordinary situation, where justice requires" the Commissioner may waive or suspend the rules and accord this application a January 12, 1996 filing date pursuant

Application No. 08/585,895

Page 2

to 37 CFR 1.183. In this case, petitioners request waiver of the rules under 37 CFR 1.183 based on the need to protect applicants' patent rights in foreign countries.

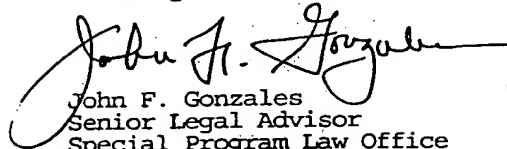
Under the circumstances of this particular case, it is deemed appropriate to waive the rules pursuant to 37 CFR 1.183 in order to accord the application a filing date of January 12, 1996.

The petition under 37 CFR 1.183 is granted.

Receipt is acknowledged of the combined declaration and power of attorney filed April 1, 1996.

The application is being returned to Application Processing Division for further processing with a filing date of January 12, 1996.

Any inquiries related to this decision should be directed to Tim Heitbrink at (703) 308-6713, or if not available, to the undersigned at (703) 305-9282.



John F. Gonzales
Senior Legal Advisor
Special Program Law Office
Office of the Deputy Assistant Commissioner
for Patent Policy and Projects

twh

Enclosure: 1183 OG 60
1158 OG 8



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.

Serial No: 08/585,895

Filed: January 12, 1996

Title: RECEPTOR LIGAND

Group Art Unit: Not yet assigned

Examiner: Not yet assigned

I hereby certify that this paper is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C., 20231 on this date:

Date: August 12, 1996

David A. Gass
David A. Gass
Registration No. 38,153
Attorney for Applicants

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

The Applicants respectfully request entry of this Preliminary Amendment prior to examination of the above-identified application on the merits by the Patent and Trademark Office.

AMENDMENTS

In the Specification:

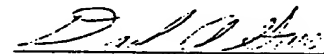
At page 1, line 3, after "August 1, 1995.", please insert -- This application is also a continuation-in-part of U.S. Patent Application Serial No. 08/340,011, filed November 14, 1994.--

REMARKS

The specification has been amended herein to claim priority from an earlier-filed U.S. application. This amendment is accompanied by a supplemental inventors' declaration which acknowledges this priority claim.

Respectfully submitted,

Dated: Aug. 10, 1973



David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

MP 1204
PATENT APPLICATION
28113/33072 #

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitalo, Kari)
and Joukov, Vladimir)
Serial No.: 08/585,895)
Filed: January 12, 1996)
For: "Receptor Ligand")
Group Art Unit: 1806)
Examiner: To be determined)

I hereby certify that this paper and
the documents referred to as enclosed
herewith are being deposited with the
United States Postal Service as First
Class Mail, postage prepaid, in an
envelope addressed to: Assistant
Commissioner for Patents,
Washington, DC 20231, on this date:
October 14, 1996
David A. Gass
David A. Gass
Reg. No.: 38,153
Attorney for Applicants

**INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of each listed document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

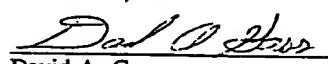
Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

October 14, 1996

By: David A. Gass
David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitalo, Kari)	I hereby certify that this paper and
and Joukov, Vladimir)	the documents referred to as enclosed
Serial No.: 08/585,895)	herewith are being deposited with the
)	United States Postal Service as First
)	Class Mail, postage prepaid, in an
)	envelope addressed to: Assistant
Filed: January 12, 1996)	Commissioner for Patents,
)	Washington, DC 20231, on this date:
For: "Receptor Ligand")	October 14, 1996
Group Art Unit: 1806)	
)	David A. Gass
)	Reg. No.: 38,153
Examiner: To be determined)	Attorney for Applicants

**INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of each listed document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

October 14, 1996

By: 

David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Driv
Chicago, Illinois 60606-6402
(312) 474-6300



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
--------------------	-------------	-----------------------	---------------------

08/585,895 01/12/96 ALVIN LO

28113/33072

1982/1125
MARSHALL O'TOOLE GERSTEIN MURRAY & BORUM
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

EXAMINER

LATHROP, B

ART UNIT	PAPER NUMBER
----------	--------------

1801

6

DATE MAILED:

11/25/96

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

- ☐ Responsive to communication(s) filed on _____
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 0 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

- ☒ Claim(s) 1-16 is/are pending in the application.
- Of the above, claim(s) _____ is/are withdrawn from consideration.
- ☐ Claim(s) _____ is/are allowed.
- ☐ Claim(s) _____ is/are rejected.
- ☐ Claim(s) _____ is/are objected to.
- ☒ Claims 1-16 are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been received.
- ☐ received in Application No. (Series Code/Serial Number) _____
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

- ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☐ Notice of Reference Cited, PTO-892
- ☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Notice of Informal Patent Application, PTO-152

- SEE OFFICE ACTION ON THE FOLLOWING PAGES -

Serial Number: 08/585895
Art Unit: 1801

2

DETAILED ACTION

Election/Restriction

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:
 - I. Claims 1, 2, 8-10, 12, and 16, drawn to a ligand for the Flt4 receptor and compositions comprising the same, classified in class 530, subclass 399.
 - II. Claims 3-7, 11, and 13, drawn to nucleic acids encoding a ligand for the Flt4 receptor and vectors and host cells comprising the same, classified in class 536, subclass 23.51.
 - III. Claims 14-15, drawn to an antibody specifically reactive to a ligand for the Flt4 receptor, classified in class 530, subclass 387.1.
2. The inventions are distinct, each from the other because of the following reasons:
3. The protein of Group I is a patentably distinct chemical species from the nucleic acids of Group II, although related as the nucleic acids encode the protein. The protein can be made without recourse to the nucleic acids by the materially distinct process of biochemical purification from tissue or serum, and the nucleic acids have separate utility as probes for screening expression libraries.
4. The protein of Group I is a patentably distinct chemical species from the antibody of Group III, although related as the antibody can bind the protein. The antibody can cross-react with other proteins, and other antibodies can cross-react with the protein. The protein can be

Serial Number: 08/585895
Art Unit: 1801

3

made without recourse to the antibody by the materially distinct process of biochemical purification from tissue or serum, and the protein has separate utility as a therapeutic agent.

5. The antibodies of Group III are patentably distinct from the nucleic acids of Group II, although related as the antibodies may be raised against proteins encoded by the nucleic acids. The inventions have distinct chemical compositions and distinct functions. The nucleic acids are not required to make the antibodies, which may be raised against proteins made without recombinant expression. The nucleic acids have separate utility as probes, for example.

6. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, restriction for examination purposes as indicated is proper.

7. A telephone call was made to David Gass on 14 November 1996 to request an oral election to the above restriction requirement, but did not result in an election being made.

Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

8. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(h).

Serial Number: 08/585895
Art Unit: 1801

4

Conclusion

9. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop, whose phone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

The examiner will attempt to respond to voice messages within 24 hours. Alternately, the examiner's supervisor, Vasu Jagannathan, can be reached at (703) 306-2777. The FAX number for Art Unit 1801 is (703) 305-7401.

An inquiry of a general nature relating to the status of this application should be directed to the Group 1800 receptionist whose telephone number is (703) 308-0196.

Brian K. Lathrop, Ph.D.

Art Unit 1801

Vasu Jagannathan
SPE
AU 1801

1806

038° T/8

PATENT #
28113/33072 G
12-1
pre/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.)	I hereby certify that this paper is
)	being deposited with the United
Serial No: 08/585,895)	States Postal Service with sufficient
)	postage as first class mail in an
Filed: January 12, 1996)	envelope addressed to: Assistant
)	Commissioner for Patents,
Title: RECEPTOR LIGAND)	Washington, D.C., 20231 on this
)	date:
Group Art Unit: Not yet assigned)	
)	Date: <u>August 12, 1996</u>
Examiner: Not yet assigned)	
)	<u>David A. Gass</u>
)	David A. Gass
)	Registration No. 38,153
)	Attorney for Applicants

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

The Applicants respectfully request entry of this Preliminary
Amendment prior to examination of the above-identified application on the merits
by the Patent and Trademark Office.

AMENDMENTS

In the Specification:

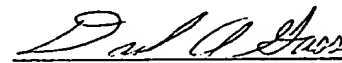
At page 1, line 3, after "August 1, 1995.", please insert This
A₁ application is also a continuation-in-part of U.S. Patent Application Serial No.
08/340,011, filed November 14, 1994.

REMARKS

The specification has been amended herein to claim priority from an earlier-filed U.S. application. This amendment is accompanied by a supplemental inventors' declaration which acknowledges this priority claim.

Respectfully submitted,

Dated: August 12, 1996



David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):)	Title: Receptor Ligand
)	
Alitalo et al.)	
)	
Serial No: 08/585,895)	Group Art Unit: Not yet assigned
)	
Filed: January 12, 1996)	Examiner: Not yet assigned
)	

TRANSMITTAL LETTER


*Assistant Commissioner for Patents
Washington, D.C. 20231*

Sir:

Transmitted herewith are a Preliminary Amendment and an executed Inventors' Declaration for the above application.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 12, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

- ☐ Verified statement(s) claiming small entity status is(are) attached.
☐ Small entity status has been established and is still effective.
☒ Has not been established.

2. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

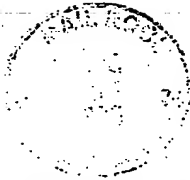
Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: August 12, 1996

By: 

David A. Gass
Reg. No: 38,153



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):)	Title: Receptor Ligand
)	
Alitalo et al.)	
)	
Serial No: 08/585,895)	Group Art Unit: Not yet assigned
)	
Filed: January 12, 1996)	Examiner: Not yet assigned
)	

TRANSMITTAL LETTER

***Assistant Commissioner for Patents
Washington, D.C. 20231***

Sir:

Transmitted herewith are a Preliminary Amendment and an executed Inventors' Declaration for the above application.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 12, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

- ☐ Verified statement(s) claiming small entity status is(are) attached.
☐ Small entity status has been established and is still effective.
☒ Has not been established.

2. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: August 13, 1996

By: 

David A. Gass
Reg. No: 38,153



Atty. Docket No: 28113/33072

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a herein named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed as Application Serial No. 08/585,895. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment attached hereto. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Priority Claimed
☐ ☒

950624	Finland	13 February 1995
(Application Serial Number)	(Country)	(Day/Month/Year Filed)

Yes No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

(Application Serial Number)	(Day/Month/Year Filed)
-----------------------------	------------------------

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

08/340,011	14 November 1994	Pending
(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)
08/510,133	01 August 1995	Pending
(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shulman (19,412)
Donald J. Brott (19,490)
Owen J. Murray (22,111)
Allen H. Gerstein (22,218)
Nate F. Scarpelli (22,320)
Edward M. O'Toole (22,477)
Michael F. Borun (25,447)

Trevor B. Joika (25,542)
Timothy J. Vezau (26,348)
Carl E. Moore, Jr. (26,487)
Richard H. Anderson (26,526)
Patrick D. Ertel (26,877)
James P. Zeller (28,491)
William E. McCracken (30,195)

Richard A. Schurr (30,890)
Anthony Nimmo (30,920)
Christine A. Dudzik (31,245)
Kevin D. Hogg (31,839)
Jeffrey S. Sharp (31,879)
Donald J. Pochopien (32,167)
Martin J. Hirsch (32,237)

James J. Napoli (32,361)
Richard M. La Barge (32,254)
Jeffrey W. Smith (33,455)
Douglass C. Hochstetler (33,710)
Cynthia L. Schaller (34,245)
Robert M. Gerstein (34,824)
David A. Gass (38,153)

Send correspondence to: David A. Gass

FIRM NAME	PHONE NO.	STREET	CITY & STATE	ZIP CODE
Marshall, O'Toole, Gerstein, Murray & Borun	312-474-6300	6300 Sears Tower 233 South Wacker Drive	Chicago, Illinois	60606-6402

Full Name of First or Sole Inventor	Citizenship
Kari Alitalo	Finland
Residence Address - Street	Post Office Address - Street
Nyyrikintie 4A	Same
City (Zip)	City (Zip)
02100 Espoo	Same
State or Country	State or Country
FINLAND <i>Fix</i>	Same
Date	Signature
<i>Aug 6, 1996</i>	<i>[Signature]</i>

☒ See second page for additional inventor

See reverse for relevant rules & statutes

Second Joint Inventor, if any Vladimir Joukov	Citizenship Russia
Residence Address - Street Topeliuksenkatu 32G8	Post Office Address - Street Same
City (Zip) 00290 Helsinki	City (Zip) Same
State or Country FINLAND <i>FX</i>	State or Country Same
Date ☐ Aug. 6, 1996	Signature ☐ <i>V. Joukov</i>

*see #420
in 1996
change*

69652 U.S. '0



12/23/96

#9
1-13-97
mu

PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:)	I hereby certify that this paper and the
)	documents referred to as enclosed
Alitalo et al.)	herewith are being deposited with th
)	United States Postal Service as First
Serial No.: 08/585,895)	Class Mail, postage prepaid, in an
)	envelope addressed to: Assistant
Filed: January 12, 1996)	Commissioner for Patents, Washington,
)	DC 20231, on this date:
For: "Receptor Ligand")	
)	Date: <u>December 19, 1996</u>
Group Art Unit: 1801)	<u>David A. Gass</u>
)	David A. Gass
Examiner: Lathrop, B.)	Reg. No.: 38,153
)	Attorney for Applicant

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

RECEIVED
JAN 10 1997
GROUP 1800

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of the listed documents are enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by

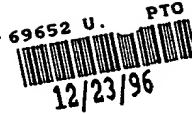
the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: December 19, 1996

By: David A. Gass
David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:)	I hereby certify that this paper and th
Alitalo et al.)	documents referred to as enclosed
Serial No.: 08/585,895)	herewith are being deposited with the
Filed: January 12, 1996)	United States Postal Service as First
For: "Receptor Ligand")	Class Mail, postage prepaid, in an
Group Art Unit: 1801)	envelope addressed to: Assistant
Examiner: Lathrop, B.)	Commissioner for Patents, Washington,
)	DC 20231, on this date:
)	Date: <u>December 19, 1996</u>
)	<u>David A. Gass</u>
)	David A. Gass
)	Reg. No.: 38,153
)	Attorney for Applicant

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

RECEIVED
JAN 10 1997
GROUP 1800

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of the listed documents are enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by

the Patent Office without payment of a fee. See 37 C.F.R. 51.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: December 19, 1996

By: David A. Gass
David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



#34201
I.D.S. 4/29/97
PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:)	I hereby certify that this paper and the
Alitalo et al.)	documents referred to as enclosed
Serial No.: 08/585,895)	herewith are being deposited with the
Filed: January 12, 1996)	United States Postal Service as First
For: "Receptor Ligand")	Class Mail, postage prepaid, in an
Group Art Unit: 1801)	envelope addressed to: Assistant
Examiner: Lathrop, B.)	Commissioner for Patents, Washington,
)	DC 20231, on this date:
)	Date: <u>21 January 1997</u>
)	<u>David A. Gass</u>
)	David A. Gass
)	Reg. No.: 38,153
)	Attorney for Applicant

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of each listed document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by

the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: 21 Jan. 1997

By: David A. Gass

David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:)	I hereby certify that this paper and th
Alitalo et al.)	documents referred to as enclosed
Serial No.: 08/585,895)	herewith are being deposited with the
Filed: January 12, 1996)	United States Postal Service as First
For: "Receptor Ligand")	Class Mail, postage prepaid, in an
Group Art Unit: 1801)	envelope addressed to: Assistant
Examiner: Lathrop, B.)	Commissioner for Patents, Washington,
)	DC 20231, on this date:
)	Date: <u>21 January 1997</u>
)	<u>David A. Gass</u>
)	David A. Gass
)	Reg. No.: 38,153
)	Attorney for Applicant

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of each listed document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by

the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: 21 Jan. 1997

By:



David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No. 08/585,895

Filed: January 12, 1996

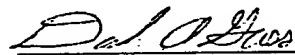
For: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

I hereby certify that this paper is
being deposited with the United
States Postal Service as first class
mail, postage prepaid, in an
envelope addressed to: Assistant
Commissioner for Patents,
Washington, D.C. 20231, on this
date:

Dated: 24 Jan. 1997


David A. Gass

AMENDMENT AND ELECTION IN RESPONSE TO RESTRICTION REQUIREMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In an official communication dated November 25, 1996, the U.S. Patent and Trademark Office issued a restriction requirement in the above-identified patent application, and set a 30 day period for response. This amendment and election in response to the restriction requirement has been timely filed with a petition for one month extension of time and petition fee, extending the time for response until January 25, 1997.

RECEIVED
JAN 27 1997
PAT. & TRADEMARK OFFICE

AMENDMENTS

In the claims:

Please cancel claims 2, 8-10, 12, and 14-16 without prejudice; amend claims 1, 3, 5, 11, and 13; and add new claims 17-25 to the application as shown below.

B1 sub
C4

1. (Amended) A purified and isolated polynucleotide encoding a polypeptide which specifically binds to the Flt4 receptor tyrosine kinase.

B2 sub
C5

3. (Amended) A purified and isolated nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 33. [the peptide according to claim 2.]

B3 sub
C5

5. (Amended) A vector comprising the nucleic acid according to claim 3 [4].

B4 sub
C7

11. (Amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises approximately amino acids 1 to 120 of SEQ ID NO: 33. [encoding the fragment of claim 10.]

B5

13. (Amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises approximately amino acids 1 to 180 of SEQ ID NO: 33. [encoding the fragment of claim 12.]

B6

--17. A host cell transformed or transfected with a vector according to claim 5.

sub
C8

18. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33, said portion encoding a polypeptide capable of binding to an Flt4 receptor tyrosine kinase.

Sub
D9
B6
sub
E9

19. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide is capable of stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase.

20. A purified and isolated nucleic acid according to claim 19 wherein said polypeptide has an apparent molecular weight of about 23 kd as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

21. A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises an amino-terminal amino acid sequence set forth in SEQ ID NO: 13.

22. A purified and isolated nucleic acid according to claim 21 wherein said polypeptide comprises approximately 120 amino acids.

23. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

24. A vector comprising a nucleic acid according to claim 18.

25. A host cell transformed or transfected with a vector according to claim 24.--

REMARKS

This is the Applicants' second amendment to this application. A preliminary amendment, to add a priority claim to the application, was mailed on August 12, 1996.

The Applicants do not intend by the foregoing amendments or any other amendments to abandon the subject matter of any claim as originally filed or later amended, and reserve the right to claim such subject matter in other

applications, such as continuations, continuations-in-part, and divisional applications.

I. The Applicants Elect Claims 3-7, 11, and 13 (Group II) without traverse.

In response to the restriction requirement, the Applicants hereby elect Group II (Claims 3-7, 11, and 13), drawn to nucleic acids, vectors, and host cells.

II. Explanation of amendments.

Claim 1 has been amended to recite a nucleic acid, rather than a polypeptide, thereby bringing claim 1 within the scope of the elected invention of Group II.

Claim 3, which formerly depended from non-elected claim 2, has been amended to be an independent claim. Claims 11 and 13 have been amended similarly.

New claims 18 and 19 find support in Example 4 (pp. 19-21), for example.

New claim 20 finds support in Example 5 (p. 22, lines 33-34), for example.

New claim 21 finds support in Example 5 at p. 23, lines 9-10, for example.

New claim 22 finds support at p. 23, lines 9-10, and p. 30, lines 14-17, for example.

New claim 23 finds support in Example 13 (p. 31, lines 33-34), for example.


All of the pending claims (as amended herein) are properly classified with the Group II claims elected by the Applicants in response to the restriction requirement. None of the new claims introduce new matter.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: 24 Jan. 1997

By:


David A. Gass
Reg. No: 38,153



H. 14
E.H. 047.6111E
4/29/97

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):)	Title: RECEPTOR LIGAND
Alitalo et al.)	
Serial No: 08/585,895)	Group Art Unit: 1801
Filed: January 12, 1996)	Examiner: Lathrop, B.

**AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME**

*Assistant Commissioner for Patents
Washington, D.C. 20231*


Sir:

Transmitted herewith is an amendment for the above application.

C 010 1 00585895 00210 870100 870204 115 110.00

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on January 24, 1997, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

- ☐ Verified statement(s) claiming small entity status is(are) attached.
- ☐ Small entity status has been established and is still effective.
- ☒ Has not been established.

2. **Extension of Time**

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month	x	\$110.00		\$55.00
Two Months		\$390.00		\$195.00
Three Months		\$930.00		\$465.00
Four Months		\$1,470.00		\$735.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$110.00

- ☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$_____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$_____

Extension Fee Due With This Request \$110.00

3. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	17	MINUS	20	= 0	X11 =	\$	X22 =	\$
INDEP.	3	MINUS	3	= 0	X40 =	\$	X80 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim					+130 =	\$	+260 =	\$
TOTAL ADDITIONAL FEE						\$	OR	\$00.00

4. **Method of Payment of Fees**

- ☒ Attached is a check in the amount of: \$110.00
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

5. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: 24 Jan. 1997

By: David A. Gass
David A. Gass
Reg. No: 38,153

1800



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):)	Title: RECEPTOR LIGAND
)	
Alitalo et al.)	
)	
Serial No: 08/585,895)	Group Art Unit: 1801
)	
Filed: January 12, 1996)	Examiner: Lathrop, B.
)	

**AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME**

*Assistant Commissioner for Patents
Washington, D.C. 20231*

Sir:

Transmitted herewith is an amendment for the above application.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on January 24, 1997, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

- ☐ Verified statement(s) claiming small entity status is(are) attached.
- ☐ Small entity status has been established and is still effective.
- ☒ Has not been established.

2. **Extension of Time**

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month	x	\$110.00		\$55.00
Two Months		\$390.00		\$195.00
Three Months		\$930.00		\$465.00
Four Months		\$1,470.00		\$735.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$110.00

- ☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$_____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$_____

Extension Fee Due With This Request \$110.00

3. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	17	MINUS	20	= 0	X11 =	\$	X22 =	\$
INDEP.	3	MINUS	3	= 0	X40 =	\$	X80 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim					+ 130 =	\$	+ 260 =	\$
TOTAL ADDITIONAL FEE						\$	OR	\$100.00

4. **Method of Payment of Fees**

- ☒ Attached is a check in the amount of: \$110.00
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

5. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: 24 Jan, 1997

By: David A. Gass
David A. Gass
Reg. No: 38,153





Gp. 1301

PATENT APPLICATION
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

The Application of: Alitalo, Kari
and Joukov, Vladimir

Serial No.: 08/585,895

Filed: January 12, 1996

For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.

#11

"EXPRESS MAIL"
Mailing label No. EMO99898621US
Date of Deposit: February 11, 1997
I hereby certify that this paper and the
documents referred to as enclosed herewith are
being deposited with the United States Postal
Service "EXPRESS MAIL POST OFFICE TO
ADDRESSEE" service under 37 CFR §1.10 on
the date indicated above and is addressed to:
Assistant Commissioner for Patents,
Washington, D.C. 20231
Mark Bonadonna
Mark Bonadonna

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed documents, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of each document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Feb 11, 1997

By: *David A. Gass*
David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

RECEIVED
MAR 21 1997
GROUP 1800

PATENT APPLICATION
23967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitalo, RUDOLPH
and Joukov, Vladimir

Serial No.: 08/585,895

Filed: January 12, 1996

For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.



) "EXPRESS MAIL"
) Mailing label No. EMO99898621US
) Date of Deposit: February 11, 1997
) I hereby certify that this paper and the
) documents referred to as enclosed herewith are
) being deposited with the United States Postal
) Service "EXPRESS MAIL POST OFFICE TO
) ADDRESSEE" service under 37 CFR §1.10 on
) the date indicated above and is addressed to:
) Assistant Commissioner for Patents,
) Washington, D.C. 20231
) *Mark Bonadonna*
) Mark Bonadonna

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

RECEIVED
MAR 21 1997
GROUP 12

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed documents, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of each document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Feb 11, 1997

By: *David A. Gass*
David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Form PTO-1449 (Modified)

U.S. Department of Commerce
Patent and Trademark OfficeAtty. Docket No.
28967/33072Serial No.
08/585,895

INFORMATION DISCLOSURE STATEMENT

Applicant
Alitalo and JoukovFiling Date
01/12/96Group
1801

(Use several sheets if necessary)

U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate

FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

<i>BA</i>	C111	Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors and Receptors Involved in Angiogenesis," <i>The 9th International Conference of the International Society of Differentiation (ISD), Development, Cell Differentiation and Cancer</i> , Pisa (Italy), September 28-October 2, 1996, p. 66 (ABSTRACT S22).
	C112	Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors B and C and Receptors Involved in Angiogenesis," <i>German-American Academic Council Foundation (GAAC)/ Stiftung Deutsch-Amerikanisches Akademisches Konzil (DAAK), 2nd Symposium on Current Problems in Molecular Medicine: The Role of Cytokines in Human Disease</i> , November 17-20, 1996, Ringberg Castle, Germany, p. 1 (ABSTRACT).
	C113	Kukk <i>et al.</i> , "VEGF-C Receptor Binding and Pattern of Expression with VEGFR-2 Suggests a Role in Lymphatic Vascular Development," <i>Development</i> , 122:3829-3837 (1996).
	C114	Paavonen <i>et al.</i> , "Chromosomal Localization and Regulation of Human Vascular Endothelial Growth Factors B and C (VEGF-B and VEGF-C)," <i>IX International Vascular Biology Meeting</i> , Seattle, Washington, September 4-8, 1996, p. 76 (ABSTRACT 299).


EXAMINER

Brian Lathrop

DATE CONSIDERED

5/2/97

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Form PTO-1449 (Modified)		U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28967/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)			Applicant Alitalo and Joukov	
			Filing Date 01/12/96	Group 1801

U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate

FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No

OTHER DOCUMENTS (including Author, Title, Date, Pertinent Pages, etc.)

	C111	Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors and Receptors Involved in Angiogenesis," <i>The 9th International Conference of the International Society of Differentiation (ISD), Development, Cell Differentiation and Cancer</i> , Pisa (Italy), September 28-October 2, 1996, p. 66 (ABSTRACT S22).
<i>Page 1</i>	C112	Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors B and C and Receptors Involved in Angiogenesis," <i>German-American Academic Council Foundation (GAAC)/ Stiftung Deutsch-Amerikanisches Akademisches Konzil (DAAK), 2nd Symposium on Current Problems in Molecular Medicine: The Role of Cytokines in Human Disease</i> , November 17-20, 1996, Ringberg Castle, Germany, p. 1 (ABSTRACT).
	C113	Kukk <i>et al.</i> , "VEGF-C Receptor Binding and Pattern of Expression with VEGFR- Suggests a Role in Lymphatic Vascular Development," <i>Development</i> , 122:3829-3837 (1996).
	C114	Paavonen <i>et al.</i> , "Chromosomal Localization and Regulation of Human Vascular Endothelial Growth Factors B and C (VEGF-B and VEGF-C)," <i>IX International Vascular Biology Meeting</i> , Seattle, Washington, September 4-8, 1996, p. 76 (ABSTRACT 299).

EXAMINER

DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.



Ep 1801
PATENT APPLICATION #
28967/33072

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996

For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this date:

Dated: March 21, 1997

David A. Gass
David A. Gass
Registration No. 38,153
Attorney for Applicant

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

RECEIVE
APR 10 1997
GROUP 1801

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed document, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of the document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: March 21, 1997

By: David A. Gass

David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



PATENT APPLICATION
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996


For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this date:

Dated: March 21, 1997


David A. Gass
Registration No. 38,153
Attorney for Applicant

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

RECEIVED
APR 10 1997
GROUP 1800

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed document, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of the document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: March 21, 1997

By: 

David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Form PTO-1449 (Modified)

U.S. Department of Commerce
Patent and Trademark OfficeAtty. Docket No.
28967/33072Serial No.
08/585,895

INFORMATION DISCLOSURE STATEMENT

(Use several sheets if necessary)

Applicant
Alitalo and JoukovFiling Date
01/12/96Group
1801

U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate

FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

<i>Cell</i>	C115	Pajusola, "Cloning and Characterization of a New Endothelial Receptor Tyrosine Kinase Flt4 and Two Novel VEGF-Like Growth Factors VEGF-B and VEGF-C," Academic Dissertation, Molecular/Cancer Biology Laboratory and Department of Pathology, Haartman Institute and Department of Biosciences, Division of Genetics, University of Helsinki, (January 26, 1996)

EXAMINER *Forian Lathrop*DATE CONSIDERED *5/5/97*

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT
28967/33072

Gp 1801
#10
5/17

In the Application of:
Alitalo et al.
Serial No.: 08/585,895
Filed: January 12, 1996
For: "Receptor Ligand"
Group Art Unit: 1801
Examiner: Lathrop, B.

I hereby certify that this paper is being
deposited with the United States Postal
Service as first class mail, postage prepaid,
in an envelope addressed to: Assistant
Commissioner for Patents, Washington, D.C.
20231, on this date:
Dated: Apr 11, 1997
RECEIVED
MAY 06 1997
GROUP 1800
David A. Gass
Registration No. 38,153
Attorney for Applicant

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed documents, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of each itemized document is enclosed herewith. Both of the documents were identified in an International Search Report (ISR, copy enclosed herewith) in a related PCT patent application. Documents identified in the ISR that are not itemized on the attached Form PTO-1449 have already been made of record by the Patent Office or the applicants.

This Information Disclosure Statement is not intended to be an admission that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 37 U.S.C. §102 or §103.

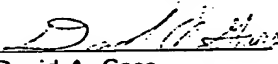
Pursuant to 37 C.F.R. §1.97(e)(1), the Applicants certify that each document itemized in the attached form PTO-1449 was cited in a

communication (an ISR) from a foreign patent office (the European Patent Office) in a counterpart foreign (PCT) application, not more than three months prior to the filing of this statement. Accordingly, pursuant to 37 C.F.R. §1.97(c)(2), the information disclosed herein should be considered by the Patent Office without payment of any fee.

However, the Patent Office is hereby authorized to charge any fees due in connection with this paper to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By: 
David A. Gass
Registration No. 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: April 16, 1977



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996


For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.

) I hereby certify that this paper is being
) deposited with the United States Postal
) Service as first class mail, postage prepaid,
) in an envelope addressed to: Assistant
) Commissioner for Patents, Washington, D.C.
) 20231, on this date:

) Dated: April 16, 1997

) 
) David A. Gass
) Registration No. 38,153
) Attorney for Applicant

**INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed documents, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of each itemized document is enclosed herewith. Both of the documents were identified in an International Search Report (ISR, copy enclosed herewith) in a related PCT patent application. Documents identified in the ISR that are not itemized on the attached Form PTO-1449 have already been made of record by the Patent Office or the applicants.

This Information Disclosure Statement is not intended to be an admission that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 37 U.S.C. §102 or §103.

Pursuant to 37 C.F.R. §1.97(e)(1), the Applicants certify that each document itemized on the attached Form PTO-1449 was cited in a



RECEIVED
MAY 9 6 1997

communication (an ~~entry~~ from a foreign patent office (the European Patent Office) in a counterpart foreign (PCT) application, not more than three months prior to the filing of this statement. Accordingly, pursuant to 37 C.F.R. §1.97(c)(2), the information disclosed herein should be considered by the Patent Office without payment of any fee.

However, the Patent Office is hereby authorized to charge any fees due in connection with this paper to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By: David A. Gass
David A. Gass
Registration No. 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: April 16, 1997

Form PTO-1449 (Modified)

U.S. Department of Commerce
Patent and Trademark OfficeAny. Docket No.
28967/33072Serial No.
08/585,895Applicant
Alitalo et al.Filing Date
01/12/96Group
1801

INFORMATION DISCLOSURE STATEMENT

(Use several sheets if necessary)

U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing D If Appropri

FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No
B7	WO 95/33772	12/14/95	PET WO	-	-		

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

B7	C116	Hillier et al., "The WashU-Merck EST Project," EMBL Database entry HS991157, accession no. H07991, July 2, 1995.

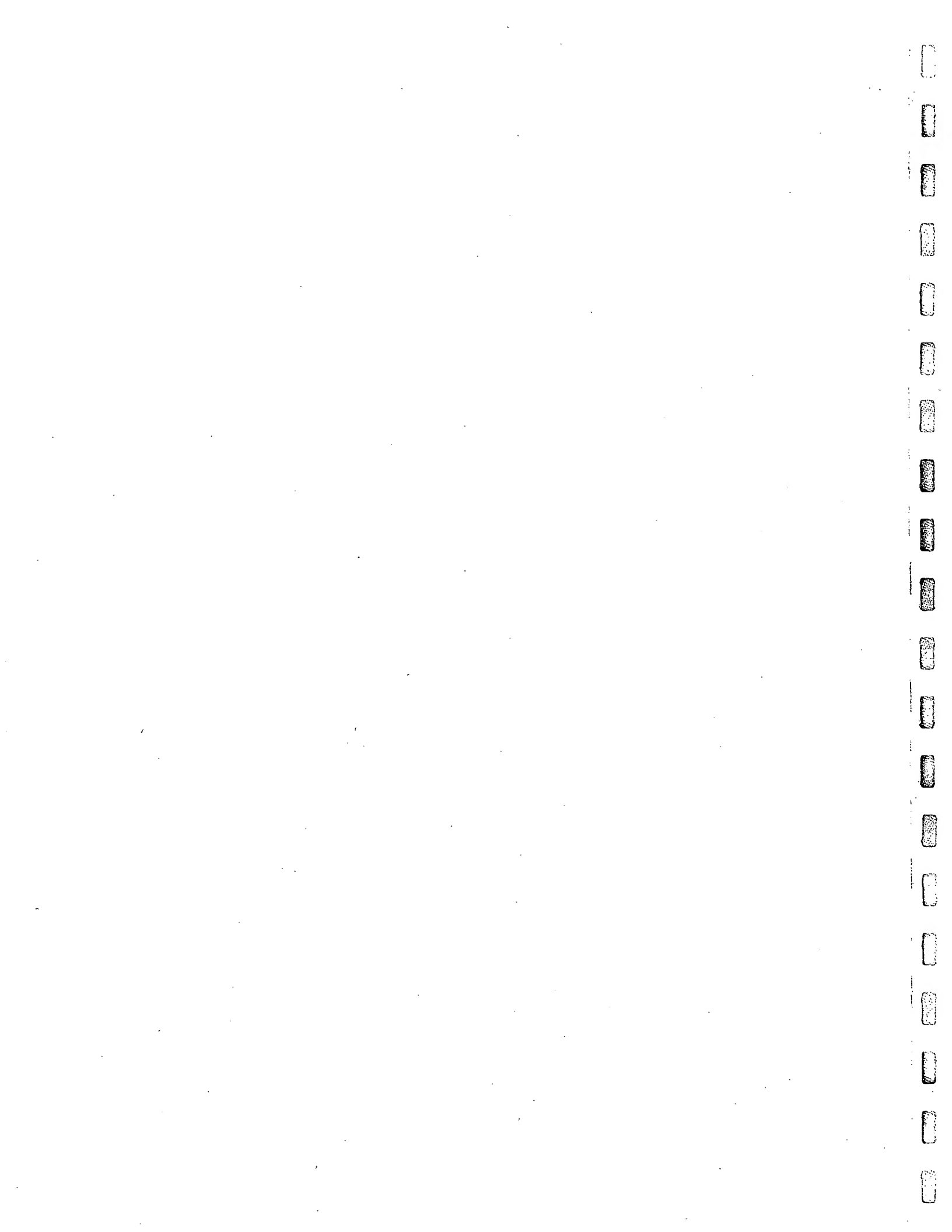
EXAMINER

Erin Lathrop

DATE CONSIDERED

5/14/97

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.





UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
--------------------	-------------	-----------------------	---------------------

EXAMINER

ART UNIT	PAPER NUMBER
----------	--------------

17

DATE MAILED:

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

☒ Responsive to communication(s) filed on 1/27/97 (Election/Amend)

☐ This action is FINAL.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) 1, 3-7, 11, 13, 17-25 is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) 1, 3-7, 11, 13, 17-25 is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☒ The specification is objected to by the Examiner.

☒ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been received.

☐ received in Application No. (Series Code/Serial Number) _____

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of Reference Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 8, 9, 11-13, 16

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

☒ Notice to Comply

- SEE OFFICE ACTION ON THE FOLLOWING PAGES -

Application No.: 08/585895; attachment to Paper No.
**NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING
NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES**

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s):

- ☒ 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.
- ☐ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c).
- ☐ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).
- ☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked -up "Raw Sequence Listing."
- ☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).
- ☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable form of the "Sequence Listing" as required by 37 C.F.R. 1.821(e).
- ☒ 7. Other: Figure 10 contains sequences requiring a SEQ ID NO, and Figure 9B requires identification of protein and polynucleotide sequences by SEQ ID NO: 32 and 33. SEQ ID NOS may be added to the Brief Description of the Drawings or the Figures.

Applicant Must Provide:

- ☒ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing".
- ☒ An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification.
- ☒ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

For questions regarding compliance to these requirements, please contact:

For Rules Interpretation, call (703) 308-4216
For CRF Submission Help, call (703) 308-4212
For PatentIn software help, call (703) 308-6856

PLEASE RETURN A COPY OF THIS NOTICE WITH YOUR RESPONSE

Serial Number: 08/585895
Art Unit: 1801

-2-

DETAILED ACTION

Election/Restriction

1. Applicant's election without traverse of Group II, claims 3-7, 11, and 13, and amendment
5 of claim 1 to read on the elected invention in Paper No. 15 is acknowledged.

Oath/Declaration

2. The oath or declaration is defective. A new oath or declaration in compliance with 37
CFR 1.67(a) identifying this application by application number and filing date is required. See
10 MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because:

Non-initialed alterations have been made to the oath or declaration. See 37 CFR 1.52(c)
and 1.57).

- 15 It does not state that the person making the oath or declaration in a continuation-in-part
application filed under the conditions specified in 35 U.S.C. 120 which discloses and
claims subject matter in addition to that disclosed in the prior copending application,
acknowledges the duty to disclose to the Office all information known to the person to be
20 material to patentability as defined in 37 CFR 1.56 which occurred between the filing date
of the prior application and the national or PCT international filing date of the
continuation-in-part application.

Serial Number: 08/585895
Art Unit: 1801

-5-

(e) the deposit will be replaced should it become necessary due to inviability, contamination, or loss of capability to function described in the manner in the specification.

5 In either case, the identifying information set forth in 37 C.F.R. 1.809(d) should be added to the specification if it is not already present. See 37 C.F.R. 1.803-1.809 for additional explanation of these requirements.

8. Claim 1 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for
10 polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for polynucleotides all polypeptides that bind the Flt4-receptor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

15 The scope of claim 1 encompasses polynucleotides from any source that encode polypeptides that bind specifically to the Flt4 receptor. Making the invention requires testing all tissues from all known species, because neither the source nor the structure of the encoded proteins are recited in the instant claims. There is no guidance provided by the specification to select those encompassed polynucleotides that encode proteins that specifically bind the Flt4
20 receptor with the exception of those teachings which support the subject matter indicated as enabled. There is no guidance to predict *a priori* whether any protein would bind the receptor without some information on the structure of the protein, and this information was simply not available for all the proteins encompassed by the claims at the time of the invention. There is no guidance provided by the state of the art to select ligands to make the invention; despite intense

Serial Number: 08/585895
Art Unit: 1801

-6-

research in this area, Borg et al. (reference C7) teach that no known ligands for the Flt4 receptor were known at the time of the invention. The amount of guidance required varies inversely with the degree of predictability involved, and in applications directed to inventions in arts where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims. MPEP 2164.03 citing *In re Soll*, 97 F.2d 623, 38 USPQ 189 (CCPA 1938) and *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970). See also *Genentech, Inc. v. Novo Nordisk A/S*, 42 USPQ2d 1001 (Fed. Cir. 1997). For the reasons set forth above, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

10

9. Claims 18-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for the scope of polynucleotides commensurate with the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

15

The breadth of claims 18-25 encompasses polynucleotides encoding all active fragments of the protein of SEQ ID NO:33, or those that are 23 kDa or 32 kDa in size. Claim 21 recites the limitation that the fragment comprises SEQ ID NO:13, but this claim encompasses polypeptides in which SEQ ID NO:13 is the only sequence derived from the protein of SEQ ID NO:33. The only

20

Serial Number: 08/585895
Art Unit: 1801

-7-

guidance offered by the specification to chose fragments that may make the invention is provided at page 11 where at least residues 1-120 of SEQ ID NO:33 are taught to be required for activity by comparison to PDGF (*infra*). Heldin et al. (reference C40), however, teach the criticality of structures throughout the corresponding region of PDGF, such as extended loop structures (Figure 2), disulfide bonds involving residue 1 (page 249, column 1), and specific residues including those up to residue 154 (*loc. cit.*). Moreover, comparison with PDGF is of limited predictive value, because the stereo-specific interaction required between VEGF-C and its receptor are different than those between PDGF and its receptor as evidenced by the fact that the two ligands do not bind the same receptors. Without additional structural information on the ligand, the skilled artisan cannot predict which additional fragments of the protein of SEQ ID NO:33 might bind the receptor. Recitation that the encoded proteins must be 23 or 32 kDa in size does not provide significant additional guidance or limitation to the scope of the claims, because this limitation does not exclude the presence of sequences unrelated to VEGF-C within the polypeptide, nor does it exclude various post-translational modifications known to profoundly influence the apparent molecular weight without affecting the primary sequence of the polypeptide. Where the art is unpredictable, as in the case of physiological activity, more guidance is required. *In re Fisher*, 166 USPQ 18 (CCPA 1970). The vast amount of experimentation required to test all the encompassed fragments is an additional factor to be considered in the overall determination of whether the experimentation required to make the invention is undue. For the reasons set forth, undue experimentation would be required to make

Serial Number: 08/585895
Art Unit: 1801

-8-

the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

10. Claims 3-5, 11, 13, and 17-25 are rejected under 35 U.S.C. 112, second paragraph, as
5 being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

SEQ ID NO:32 encodes a protein whose amino terminus is indicated as residue 1, and the specification teaches that the amino terminal of a 23 kDa protein expressed from the polynucleotide has the amino terminus shown in SEQ ID NO:13; however, Human Genome
10 Sciences, Inc, disclose DNA encoding a similar sequence (99% global identity using the Smith-Waterman algorithm with 100% identity to the instantly claimed protein in the amino terminus through the instant residue 8) whose amino terminus is indicated as residue -8 of the instant protein. It would have been understood in the art that a disclosure of a particular residue as "residue 1" would have meant that this residue was the amino terminus of the mature polypeptide;
15 however, it was unclear which residue corresponds to the amino terminus of the encoded polypeptide, making the designation of a particular amino acid residue as a "residue 1" indefinite. Although Human Genome Sciences was published after the effective filing date, the publication is used to show that the instant claims were indefinite at the time of filing. MPEP 2124 citing *In re Glass*, 492 F.2d 1228, 1232 n.6., 181 USPQ 31, 34 n.6 (CCPA 1974).

Serial Number: 08/585895
Art Unit: 1801

-9-

11. Claims 11 and 13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the term "approximately" in claims 11 and 13 is a relative term which renders the claim indefinite. The term "approximately" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

Allowable Subject Matter

12. Claims 3-5, 11, 13, and 17 would be allowable over the prior art of record if rewritten or amended to overcome the rejection(s) under 35 U.S.C. 112 set forth in this Office action.

13. The following is a statement of reasons for the indication of allowable subject matter: polynucleotides encoding the instantly claimed ligand appear to be novel over the prior art of record. Borg et al. (reference C7), for example, disclose that the ligand for Flt4 was not known in the art around the time of invention. Closest prior art is a DNA with about 99% identity to the claimed polynucleotide (Human Genome Sciences, Inc., reference B1), but the publication date antedates the effective filing date of the instant application. Other relevant prior art made of record below discloses a series of expressed sequence tags (ESTs) with high identity to large regions of the Flt4 ligand cDNA. The probable identity of these ESTs was not disclosed, and without the benefit of hindsight, the artisan at the time of invention would not have been motivated to use these ESTs to make the claimed invention. It was not known that these ESTs

Serial Number: 08/585895
Art Unit: 1801

-10-

encoded a receptor ligand, nor was it known that these ESTs were nearly identical to the Flt4 ligand cDNA at the time of invention. In fact, the only EST posited to encode a particular protein was taught to encode a Balbiani ring protein (Hillier et al., EST-STS Accession No. T81690), hardly giving motivation to use the EST to find the claimed invention.

5

Conclusion

14. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

- Eriksson et al. disclose VEGF-B and DNA encoding this protein, which appears to be structurally distinct from the instantly claimed DNAs. It does not appear that the DNAs disclosed by Eriksson et al. encode proteins that would reasonably have the inherent property of meeting the claim limitation of binding the Flt4 receptor.

10

15. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop, whose phone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

15

The examiner will attempt to respond to voice messages within 24 hours. Alternately, the examiner's supervisor, Stephen Walsh, can be reached at (703) 308-2957. The FAX number for Art Unit 1801 is (703) 305-7401.

20

An inquiry of a general nature relating to the status of this application should be directed to the Group 1800 receptionist whose telephone number is (703) 308-0196.

BKL
Brian K. Lathrop, Ph.D.
5/25/97

David L. Fitzgerald
DAVID L. FITZGERALD
PRIMARY EXAMINER
GROUP 1800

\$ 58589

NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

PTO Draftpersons review all originally filed drawings regardless of whether they are designated as formal or informal. Additionally, patent Examiners will review the drawings for compliance with the regulations. Direct telephone inquiries concerning this review to the Drawing Review Branch, 703-305-8404.

The drawings filed (insert date) 1/12/96A. not objected to by the Draftsperson under 37 CFR 1.84 or 1.152.

B. not objected to by the Draftsperson under 37 CFR 1.84 or 1.152 as indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawings must be submitted according to the instructions on the back of this Notice.

1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings:

Black ink. Color.

Not black solid lines. Fig(s) _____
Color drawings are not acceptable until petition is granted.
Fig(s) _____

2. PHOTOGRAPHS. 37 CFR 1.84(b)

Photographs are not acceptable until petition is granted.
Fig(s) _____

Photographs not properly mounted (must use bristol board or photographic double-weight paper). Fig(s) _____
Poor quality (half-tone). Fig(s) _____

3. GRAPHIC FORMS. 37 CFR 1.84(d)

Chemical or mathematical formula not labeled as separate figure.
Fig(s) _____
Group of waveforms not presented as a single figure, using common vertical axis with time extending along horizontal axis.
Fig(s) _____
Individuals waveform not identified with a separate letter designation adjacent to the vertical axis. Fig(s) _____

4. TYPE OF PAPER. 37 CFR 1.84(c)

Paper not flexible, strong, white, smooth, nonshiny, and durable.
Sheet(s) _____
Erasures, alterations, overwritings, interlineations, cracks, creases, and folds copy machine marks not accepted. Fig(s) _____
Mylar, velum paper is not acceptable (too thin). Fig(s) _____

5. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable sizes:

21.6 cm. by 35.6 cm. (8 1/2 by 14 inches)
21.6 cm. by 33.1 cm. (8 1/2 by 13 inches)
21.6 cm. by 27.9 cm. (8 1/2 by 11 inches)
21.0 cm. by 29.7 cm. (DIN size A4)

All drawing sheets not the same size. Sheet(s) _____
Drawing sheet not an acceptable size. Sheet(s) _____

6. MARGINS. 37 CFR 1.84(g): Acceptable margins:

Paper size

21.6 cm. X 35.6 cm. (8 1/2 X 14 inches)	21.6 cm. X 33.1 cm. (8 1/2 X 13 inches)	21.6 cm. X 27.9 cm. (8 1/2 X 11 inches)	21.0 cm. X 29.7 cm. (DIN Size A4)
T 5.1 cm. (2")	2.5 cm. (1")	2.5 cm. (1")	2.5 cm.
L .64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")	2.5 cm.
R .64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")	1.5 cm.
B .64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")	1.0 cm.

Margins do not conform to chart above.

Sheet(s) _____

Top (T) Left (L) Right (R) Bottom (B)

7. VIEWS. 37 CFR 1.84(h)

REMINDER: Specification may require revision to correspond to drawing changes.

All views not grouped together. Fig(s) _____
Views connected by projection lines or lead lines.
Fig(s) _____
Partial views. 37 CFR 1.84(h) 2

View and enlarged view not shown separately or properly.
Fig(s) 15, 15A

Sectional views. 37 CFR 1.84(h) 3

Hatching not indicated for sectional portions of an object.
Fig(s) _____

Cross section not drawn same as view with parts in cross section with regularly spaced parallel oblique strokes. Fig(s) _____

8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(i)

Words do not appear on a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) _____

9. SCALE. 37 CFR 1.84(k)

Scale not large enough to show mechanism with crowding when drawing is reduced in size to two-thirds in reproduction.
Fig(s) _____

Indication such as "actual size" or scale 1/2" not permitted.
Fig(s) _____

10. CHARACTER OF LINES, NUMBERS, & LETTERS. 37 CFR 1.84(j)

Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (except for color drawings).
Fig(s) 1-5, 1A

11. SHADING. 37 CFR 1.84(m)

Solid black shading areas not permitted.
Fig(s) _____

Shade lines, pale, rough and blurred. Fig(s) _____

12. NUMBERS, LETTERS, & REFERENCE CHARACTERS. 37 CFR 1.84(p)

Numbers and reference characters not plain and legible. 37 CFR 1.84(p)(1) Fig(s) 3

Numbers and reference characters not oriented in same direction as the view. 37 CFR 1.84(p)(1) Fig(s) _____

English alphabet not used. 37 CFR 1.84(p)(2)
Fig(s) _____

Numbers, letters, and reference characters do not measure at least .32 cm. (1/8 inch) in height. 37 CFR(p)(3)
Fig(s) 2, 3, 10, 11

13. LEAD LINES. 37 CFR 1.84(q)

Lead lines cross each other. Fig(s) _____
Lead lines missing. Fig(s) _____

14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(i)

Sheets not numbered consecutively, and in Arabic numerals, beginning with number 1. Sheet(s) _____

15. NUMBER OF VIEWS. 37 CFR 1.84(u)

Views not numbered consecutively, and in Arabic numerals, beginning with number 1. Fig(s) _____
View numbers not preceded by the abbreviation Fig.
Fig(s) _____

16. CORRECTIONS. 37 CFR 1.84(w)

Corrections not made from prior PTO-948.
Fig(s) _____

17. DESIGN DRAWING. 37 CFR 1.152

Surface shading shown not appropriate. Fig(s) _____
Solid black shading not used for color contrast.
Fig(s) _____

COMMENTS:

- Fig legend placed incorrectly (Fig 9A, 15A, 16A)

1.1A

9/17/96

Notice of References Cited				Application No. 08/585,895		Applicant(s) Alitalo et al.	
				Examiner Brian Lathrop		Group Art Unit 1801	
Page 1 of 1							
U.S. PATENT DOCUMENTS							
		DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS	
	A	5,607,918	3/4/97	Eriksson et al.	514	12	
	B						
	C						
	D						
	E						
	F						
	G						
	H						
	I						
	J						
	K						
	L						
	M						
FOREIGN PATENT DOCUMENTS							
		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUBCLASS
	N						
	O						
	P						
	Q						
	R						
	S						
	T						
NON-PATENT DOCUMENTS							
		DOCUMENT (Including Author, Title, Source, and Pertinent Pages)					DATE
	U	Hillier et al. y185b08.21 Homo sapiens cDNA clone 44993 5'. EST-STS Accession No. H05177.					6/21/95
	V	Hillier et al. y186g06.r1 Homo sapiens cDNA clone 45138 5'. EST-STS Accession No. H07991.					6/23/95
	W	Hillier et al. yd29f07.r1 Homo sapiens cDNA clone 109669 5' similar to SP:BAR3_CHITE Q03376 CALCIUM BINDING PROTEIN 3. EST-STS Accession No. T81690.					3/15/95
	X	Auffray et al. H. sapiens partial cDNA sequence; clone c-1w11. EST-STS Accession No. Z44272.					11/6/94

Brian Lathrop 5/25/97

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28113/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 01/12/96	Group 1806 1801

U.S. PATENT DOCUMENTS							
*Examiner Initials		Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate
BKL	A1	5,332,671	07/26/94	Ferrara <i>et al.</i>	435	240.1	
BKL	A2	5,219,739	06/15/93	Tischer <i>et al.</i>	435	69.4	

FOREIGN PATENT DOCUMENTS								
*Examiner Initials		Document Number	Publication Date	Country	Class	Subclass	Translation	
							Yes	No
BKL	B1	WO 95/24473 A1	09/14/95	PCT WO	—	—		
BKL	B2	WO 96/11269 A2	04/18/96	PCT WO	—	—		

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
BKL	C1	Andersson <i>et al.</i> , "Assignment of Interchain Disulfide Bonds in platelet-Derived Growth Factor (PDGF) and Evidence for Agonist Activity of Monomeric PDGF," <i>J. Biol. Chem.</i> , 267(16):11260-11266 (June 5, 1992).
	C2	Aprelikova <i>et al.</i> , "FLT4, A Novel Class III Receptor Tyrosine Kinase in Chromosome 5q33-qter," <i>Cancer Research</i> , 52:746-748 (February 1, 1992).
	C3	Ausprunk, <i>et al.</i> , "Migration and Proliferation of Endothelial Cells in Preformed and Newly Formed Blood Vessels during Tumor Angiogenesis," <i>Microvasc. Res.</i> , 14:53-65 (1977).
	C4	Basilico <i>et al.</i> , "The FGF Family of Growth Factors and Oncogenes," <i>Adv. Cancer Res.</i> , 59:145-165 (1992).
	C5	Berse <i>et al.</i> , "Vascular Permeability Factor (Vascular Endothelial Growth Factor) Gene is Expressed Differentially in Normal Tissues, Macrophages, and Tumors," <i>Mol. Biol. Cell.</i> , 3:211-220 (February, 1992).
	C6	Betsholtz <i>et al.</i> , "cDNA Sequence and Chromosomal Localization of Human Platelet-Derived Growth Factor A-Chain and Its Expression in Tumor Cell Lines," <i>Nature</i> , 320:695-699 (April, 1986).

EXAMINER Brian Lathrop	DATE CONSIDERED 5/8/97
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28113/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 01/12/96	Group 1806 1801

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
Borg <i>et al.</i>	C7	Borg <i>et al.</i> , "Biochemical Characterization of Two Isoforms of FLT4, a VEGF Receptor-Related Tyrosine Kinase," <i>Oncogene</i> , 10:973-84 (1995).
Breier <i>et al.</i>	C8	Breier <i>et al.</i> , "Expression of Vascular Endothelial Growth Factor During Embryonic Angiogenesis and Endothelial Cell Differentiation," <i>Development</i> , 114:521-532 (1992).
Cao <i>et al.</i>	C9	Cao <i>et al.</i> , "Heterodimers of Placenta Growth Factor/Vascular Endothelial Growth Factor," <i>J. Biol. Chem.</i> , 271(6):3154-3162 (February 9, 1996).
Cheng and Flanagan	C10	Cheng and Flanagan, "Identification and Cloning of ELF-1, A Developmentally Expressed Ligand for the Mek4 and Sek Receptor Tyrosine Kinases," <i>Cell</i> , 79:157-168 (October 7, 1994).
Claesson-Welsh <i>et al.</i>	C11	Claesson-Welsh <i>et al.</i> , "Identification and Structural Analysis of the A Type Receptor for Platelet-derived Growth Factor," <i>J. Biol. Chem.</i> , 264(3):1742-1747 (January 25, 1989).
Coffin <i>et al.</i>	C12	Coffin <i>et al.</i> , "Angioblast Differentiation and Morphogenesis of the Vascular Endothelium in the Mouse Embryo," <i>Devel. Biol.</i> , 148:51-62 (1991).
Curran and Franza	C13	Curran and Franza, "Fos and Jun: The AP-1 Connection," <i>Cell</i> , 55:395-397 (November 4, 1988).
De Vries <i>et al.</i>	C14	De Vries <i>et al.</i> , "The <i>fms</i> -Like Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor," <i>Science</i> , 255:989-991 (February 21, 1992).
Dignam <i>et al.</i>	C15	Dignam <i>et al.</i> , "Balbiani Ring 3 in <i>Chironomus tentans</i> Encodes a 185-kDa Secretory Protein Which is Synthesized Throughout the Fourth Larval Instar," <i>Gene</i> , 88:133-140 (1990).
DiSalvo <i>et al.</i>	C16	DiSalvo <i>et al.</i> , "Purification and Characterization of a Naturally Occurring Vascular Endothelial Growth Factor: Placenta Growth Factor Heterodimer," <i>J. Biol. Chem.</i> , 270(13):7717-7723 (March 31, 1995).
Don <i>et al.</i>	C17	Don <i>et al.</i> , "'Touchdown' PCR to Circumvent Spurious Priming During Gene Amplification," <i>Nucl. Acids Res.</i> , 19: 4008 (1991).
Dumont <i>et al.</i>	C18	Dumont <i>et al.</i> , "Dominant-negative and Targeted Null Mutations in the Endothelial Receptor Tyrosine Kinase, <i>tek</i> , Reveal a Critical Role in Vasculogenesis of the Embryo," <i>Genes Dev.</i> , 8:1897-1909 (1994).
Dumont <i>et al.</i>	C19	Dumont <i>et al.</i> , "Vascularization of the Mouse Embryo: A Study of <i>flk-1</i> , <i>tek</i> , <i>tie</i> and Vascular Endothelial Growth Factor Expression During Development," <i>Development Dynamics</i> , 203:80-92 (1995).

EXAMINER Brian Lathrop	DATE CONSIDERED 5/8/97
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)

U.S. Department of Commerce
Patent and Trademark OfficeAny. Docket No.
28113/33072Serial No.
08/585,895

INFORMATION DISCLOSURE STATEMENT

(Use several sheets if necessary)

Applicant
Alitalo and JoukovFiling Date
01/12/96Group
1806 (85)

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

gcl	C20	Dvorak <i>et al.</i> , "Review: Vascular Permeability Factor/Vascular Endothelial Growth Factor, Microvascular Hyperpermeability, and Angiogenesis," <i>Amer. J. Path.</i> , 146:1029-1039 (1995).
	C21	Eichmann <i>et al.</i> , "Two Molecules Related to the VEGF Receptor are Expressed in Early Endothelial Cells During Avian Embryonic Development," <i>Mech. Dev.</i> , 42:33-48 (1993).
	C22	Ferrara <i>et al.</i> , "Molecular and Biological Properties of the Vascular Endothelial Growth Factor Family of Proteins," <i>Endocrine Rev.</i> , 13(1):18-32 (1992).
	C23	Finnerty <i>et al.</i> , "Molecular Cloning of Murine FLT and FLT4," <i>Oncogene</i> , 8(11):2293-2298 (1993).
	C24	Flamme <i>et al.</i> , "Vascular Endothelial Growth Factor (VEGF) and VEGF-Receptor 2 (flk-1) are Expressed During Vasculogenesis and Vascular Differentiation in the Quail Embryo," <i>Devel. Biol.</i> , 169:699-712 (1995).
	C25	Flanagan and Leder, "The <i>kit</i> Ligand: A Cell Surface Molecule Altered in Steel Mutant Fibroblasts," <i>Cell</i> , 63:185-194 (October 5, 1990).
	C26	Folkman, "Angiogenesis in Cancer, Vascular, Rheumatoid and Other Disease," <i>Nature Med.</i> , 1(1):27-31 (1995).
	C27	Folkman <i>et al.</i> , "Angiogenesis," <i>J. Biol. Chem.</i> , 267(16):10931-10934 (June 5, 1992).
	C28	Folkman <i>et al.</i> , "Long-term Culture of Capillary Endothelial Cells," <i>Proc. Nat'l Acad. Sci., USA</i> , 76(10):5217-5221 (October, 1979).
	C29	Fong <i>et al.</i> , "Role of the Flt-1 Receptor Tyrosine Kinase in Regulating the Assembly of Vascular Endothelium," <i>Nature</i> , 376:66-70 (July 6, 1995).
	C30	Fournier <i>et al.</i> , "Mutation of Tyrosine Residue 1337 Abrogates Ligand-Dependent Transforming Capacity of the FLT4 Receptor," <i>Oncogene</i> , 11(5):921-931 (September 7, 1995).
	C31	Friesel <i>et al.</i> , "Molecular Mechanisms of Angiogenesis: Fibroblast Growth Factor Signal Transduction," <i>FASEB J.</i> , 9:919-25 (1995).
	C32	Galland <i>et al.</i> , "The <i>Flt4</i> Gene Encodes a Transmembrane Tyrosine Kinase Related to the Vascular Endothelial Growth Factor Receptor," <i>Oncogene</i> , 8:1233-1240 (1993).
	C33	Genbank S66407, "FLT4 Receptor Tyrosine Kinase Isoform FLT4 Long (3' Region, Alternatively Spliced) [Human, mRNA Partial, 216 nt].," Deposited by Pajusola <i>et al.</i> , Dated December 17, 1993.

EXAMINER

Brian Lathrop

DATE CONSIDERED

5/8/97

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28113/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 01/12/96	Group 1806 1801

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
<i>BHL</i>	C34	Genbank U48800, "Mus Musculus Vascular Endothelial Growth Factor B Precursor (VEGF-B) mRNA, Complete Cds.," Deposited by Olofsson <i>et al.</i> , Dated August 19, 1996.
	C35	Genbank X15997, "Human Vascular Permeability Factor mRNA, Complete Cds.," Deposited by Keck <i>et al.</i> , Dated June 15, 1990.
	C36	Genbank X60280, "Vector Plasmid pLTRpoly DNA.," Deposited by Maekelae, T.P., Dated July 16, 1996.
	C37	Genbank X68203, "H. sapiens mRNA for FLT4, Class III Receptor Tyrosine Kinase.," Deposited by Aprelikova, O., Dated November 30, 1993.
	C38	Genbank X94216, "Homo sapiens mRNA for VEGF-C protein," Deposited by Joukov <i>et al.</i> , Dated February 6, 1996.
	C39	Harlow <i>et al.</i> , <i>Antibodies, a Laboratory Manual</i> , Cold Spring Harbor Laboratory Press, pp. 72-137, 141-157, 287 & 321-358 (1988).
	C40	Heldin <i>et al.</i> , "Structure of Platelet-Derived Growth Factor: Implications for Functional Properties," <i>Growth Factors</i> , 8:245-252 (1993).
	C41	Joukov <i>et al.</i> , "A Novel Vascular Endothelial Growth Factor, VEGF-C, Is a Ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) Receptor Tyrosine Kinases," <i>EMBO J.</i> , 15(2):290-298 (1996).
	C42	Kaipainen <i>et al.</i> , "Expression of the FMS-Like Tyrosine Kinase 4 Gene Becomes Restricted to Lymphatic Endothelium During Development," <i>Proc. Nat'l Acad. Sci., USA</i> , 92:3566-3570 (April, 1995).
	C43	Kaipainen <i>et al.</i> , "The Related FLT4, FLT1 and KDR Receptor Tyrosine Kinases Show Distinct Expression Patterns in Human Fetal Endothelial Cells," <i>J. Exp. Med.</i> , 178:2077-2088 (December, 1993).
	C44	Kaipainen <i>et al.</i> , "Enhanced Expression of the Tie Receptor Tyrosine Kinase Messenger RNA in the Vascular Endothelium of Metastatic Melanomas," <i>Cancer Res.</i> , 54:6571-6577 (December 15, 1994).
	C45	Kozak, "An Analysis of 5'-Non-Coding Sequences from 699 Vertebrate Messenger RNAs," <i>Nucl. Acids Res.</i> 15: 8125-8148 (1987).
	C46	Lee <i>et al.</i> , "Vascular Endothelial Growth Factor-Related Protein: A Ligand and Specific Activator of the Tyrosine Kinase Receptor Flt4," <i>Proc. Nat'l Acad. Sci., USA</i> , 93:1988-1992 (March, 1996).
	C47	Leung <i>et al.</i> , "Vascular Endothelial Growth Factor Is a Secreted Angiogenic Mitogen," <i>Science</i> , 246:1306-1309 (December 8, 1989).

EXAMINER <i>Brian Lathrop</i>	DATE CONSIDERED <i>5/8/97</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28113/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 01/12/96	Group 1806

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
19	C48	Levy <i>et al.</i> , "Post-transcriptional Regulation of Vascular Endothelial Growth Factor by Hypoxia," <i>J. Biol. Chem.</i> , 271(5):2746-2753 (February 2, 1996).
	C49	Levy <i>et al.</i> , "Transcriptional Regulation of the Rat Vascular Endothelial Growth Factor Gene by Hypoxia," <i>J. Biol. Chem.</i> , 270(22):13333-13340 (June 2, 1995).
	C50	Lyman <i>et al.</i> , "Molecular Cloning of a Ligand for the flt3/ftk-2 Tyrosine Kinase Receptor: A Proliferative Factor for Primitive Hematopoietic Cells," <i>Cell</i> , 75:1157-1167 (December 17, 1993).
	C51	Maglione <i>et al.</i> , "Isolation of a Human Placenta cDNA Coding for a Protein Related to the Vascular Permeability Factor," <i>Proc. Nat'l Acad. Sci., USA</i> , 88:9267-9271 (October, 1991).
	C52	Maglione <i>et al.</i> , "Two Alternative mRNAs Coding for the Angiogenic Factor, Placenta Growth Factor (PlGF) are Transcribed from a Single Gene of Chromosome 14," <i>Oncogene</i> , 8:925-931 (1993).
	C53	Mäkelä <i>et al.</i> , "Plasmid pLTRpoly: A Versatile High-Efficiency Mammalian Expression Vector," <i>Gene</i> , 118: 293-294 (1992).
	C54	Matthews <i>et al.</i> , "A Receptor Tyrosine Kinase cDNA Isolated from a Population of Enriched Primitive Hematopoietic Cells and Exhibiting Close Genetic Linkage to c-kit," <i>Proc. Nat'l Acad. Sci., USA</i> , 88:9026-9030 (October, 1991).
	C55	Metzelaar <i>et al.</i> , "CD63 Antigen," <i>J. of Biol. Chem.</i> , 266(5):3239-3245 (February 15, 1991).
	C56	Millauer <i>et al.</i> , "Glioblastoma Growth Inhibited <i>in vivo</i> by a Dominant-Negative Flk-1 Mutant," <i>Nature</i> , 367:576-579 (February 10, 1994).
	C57	Millauer <i>et al.</i> , "High Affinity VEGF Binding and Developmental Expression Suggest Flk-1 as a Major Regulator of Vasculogenesis and Angiogenesis," <i>Cell</i> , 72:835-846 (March 26, 1993).
	C58	Mitchell <i>et al.</i> , "Transcription Factor AP-2 is Expressed in Neural Crest Cell Lineages During Mouse Embryogenesis," <i>Genes and Dev.</i> , 5:105-119 (1991).
	C59	Morgenstern <i>et al.</i> , "Advanced Mammalian Gene Transfer: High Titre Retroviral Vectors With Multiple Drug Selection Markers and a Complementary Helper-Free Packaging Cell Line," <i>Nucl. Acids Res.</i> , 18(12):3587-3595 (1990).
	C60	Mount, S.M., "A Catalogue of Splice Junction Sequences," <i>Nucl. Acids Res.</i> , 10(2):459-472 (1982).
	C61	Mustonen <i>et al.</i> , "Endothelial Receptor Tyrosine Kinases Involved in Angiogenesis," <i>J. Cell Biol.</i> , 129:895-898 (May, 1995).

EXAMINER Brian Lathrop	DATE CONSIDERED 5/8/97
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28113/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 01/12/96	Group 1806 1801

OTHER DOCUMENTS (including Author, Title, Date, Pertinent Pages, etc.)		
<i>Bill</i>	C62	Nelson and Sun, "The 50- and 58-kdalton Keratin Classes as Molecular Markers for Stratified Squamous Epithelia: Cell Culture Studies," <i>J. Cell Biol.</i> , 97:244-251 (July, 1983).
	C63	Neufeld <i>et al.</i> , "Vascular Endothelial Growth Factor and Its Receptors," <i>Prog. Growth Fact. Res.</i> , 5:89-97 (1994).
	C64	Oefner <i>et al.</i> , "Crystal Structure of Human Platelet-derived Growth Factor BB," <i>EMBO J.</i> , 11(11):3921-3926 (1992).
	C65	Oelrichs <i>et al.</i> , "NYK/FLK-1: A Putative Receptor Tyrosine Kinase Isolated from E10 Embryonic Neuroepithelium is Expressed in Endothelial Cells of the Developing Embryo," <i>Oncogene</i> , 8:11-18 (1993).
	C66	Olofsson <i>et al.</i> , "Vascular Endothelial Growth Factor B, A Novel Growth Factor for Endothelial Cells," <i>Proc. Nat'l Acad. Sci., USA</i> , 93:2576-2581 (March, 1996).
	C67	Paavonen <i>et al.</i> , "Novel Human Vascular Endothelial Growth Factor Genes VEGF-B and VEGF-C Localize to Chromosomes 11q13 and 4q34, Respectively," <i>Circulation</i> 93(6):1079-1082 (March 15, 1996).
	C68	Pajusola <i>et al.</i> , "FLT4 Receptor Tyrosine Kinase Contains Seven Immunoglobulin-Like Loops and Is Expressed in Multiple Human Tissues and Cell Lines," <i>Cancer Res.</i> , 52:5738-5743 (October 15, 1992).
	C69	Pajusola <i>et al.</i> , "Signalling Properties of FLT4, a Proteolytically Processed Receptor Tyrosine Kinase Related to Two VEGF Receptors," <i>Oncogene</i> , 9:3545-3555 (1994).
	C70	Pajusola <i>et al.</i> , "Two Human FLT4 Receptor Tyrosine Kinase Isoforms With Distinct Carboxy Terminal Tails are Produced by Alternative Processing of Primary Transcripts," <i>Oncogene</i> , 8: 2931-2937 (1993).
	C71	Park <i>et al.</i> , "Placenta Growth Factor, Potentiation of Vascular Endothelial Growth Factor Bioactivity In vitro and In vivo, and High Affinity Binding to Flt-1 but not to Flk-1/KDR," <i>J. Biol. Chem.</i> , 269(41):25646-25654 (October 14, 1994).
	C72	Partanen <i>et al.</i> , "A Novel Endothelial Cell Surface Receptor Tyrosine Kinase with Extracellular Epidermal Growth Factor Homology Domains," <i>Mol. & Cell. Biol.</i> , 12(4):1698-1707 (April, 1992).
	C73	Partanen <i>et al.</i> , "Putative Tyrosine Kinases Expressed in K-562 Human Leukemia Cells," <i>Proc. Nat'l Acad. Sci., USA</i> , 87:8913-8917 (November, 1990).
	C74	Paulsson <i>et al.</i> , "The Balbiani Ring 3 Gene in <i>Chironomus tentans</i> has a Diverged Repetitive Structure Split by Many Introns," <i>J. Mol. Biol.</i> , 211:331-349 (1990).

EXAMINER <i>Brian Cathrop</i>	DATE CONSIDERED <i>5/8/97</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28113/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary) 21		Applicant Alitalo and Joukov	
		Filing Date 01/12/96	Group 1806 1801

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
BUL	C75	Pear <i>et al.</i> , "Production of High-titer Helper-free Retroviruses by Transient Transfection," <i>Proc. Nat'l Acad. Sci., USA</i> , 90:8392-8396 (September, 1993).
	C76	Pertovaara <i>et al.</i> , "Vascular Endothelial Growth Factor Is Induced in Response to Transforming Growth Factor- β in Fibroblastic and Epithelial Cells," <i>J. Biol. Chem.</i> , 269(9):6271-6274 (March 4, 1994).
	C77	Peters <i>et al.</i> , "Vascular Endothelial Growth Factor Receptor Expression during Embryogenesis and Tissue Repair Suggests a Role in Endothelial Differentiation and Blood Vessel Growth," <i>Proc. Nat'l Acad. Sci., USA</i> , 90:8915-8918 (October, 1993).
	C78	Pötgens <i>et al.</i> , "Covalent Dimerization of Vascular Permeability Factor/Vascular Endothelial Growth Factor Is Essential for Its Biological Activity," <i>J. Biol. Chem.</i> , 269(52):32879-32885 (December 30, 1994).
	C79	Puri <i>et al.</i> , "The Receptor Tyrosine Kinase TIE is Required for Integrity and Survival of Vascular Endothelial Cells," <i>EMBO J.</i> , 14:5884-5891 (1995).
	C80	Quinn <i>et al.</i> , "Fetal Liver Kinase 1 is a Receptor for Vascular Endothelial Growth Factor and is Selectively Expressed in Vascular Endothelium," <i>Proc. Nat'l Acad. Sci., USA</i> , 90:7533-7537 (August, 1993).
	C81	Risau <i>et al.</i> , "Changes on the Vascular Extracellular Matrix During Embryonic Vasculogenesis and Angiogenesis," <i>Devel. Biol.</i> , 125:441-450 (1988).
	C82	Risau <i>et al.</i> , "Platelet-Derived Growth Factor is Angiogenic <i>In Vivo</i> ," <i>Growth Factors</i> , 7:261-266 (1992).
	C83	Risau, W., "Differentiation of Endothelium," <i>FASEB J.</i> , 9:926-933 (1995).
	C84	Sabin, F.R., "The Lymphatic System in Human Embryos, With A Consideration of the Morphology of the System as a Whole," <i>Am. J. Anat.</i> , 9(1):43-91 (1909).
	C85	Saksela <i>et al.</i> , "Cell-Associated Plasminogen Activation: Regulation and Physiological Function," <i>Ann. Rev. Cell Biol.</i> , 4:93-126 (1988).
	C86	Sambrook <i>et al.</i> , <i>Molecular Cloning: a Laboratory Manual</i> , Second Edition, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory (1989), pp. 2.60-2.79, 4.21-4.32, 7.3-7.36, and 9.47-9.51.
	C87	Sato <i>et al.</i> , "Distinct Roles of the Receptor Tyrosine Kinases Tie-1 and Tie-2 in Blood Vessel Formation," <i>Nature</i> , 376:70-74 (July 6, 1995).
	C88	Schneider <i>et al.</i> , "A One-step Purification of Membrane Proteins Using a High Efficiency Immunomatrix," <i>J. Biol. Chem.</i> , 257(18):10766-10769 (September 25, 1982).

EXAMINER Brian Lathrop	DATE CONSIDERED 5/8/97
<p>*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.</p>	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28113/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 01/12/96	Group #806 1801

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
Bill	C89	Seetharam <i>et al.</i> , "A Unique Signal Transduction from FLT Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor VEGF," <i>Oncogene</i> , 10:135-147 (1995).
	C90	Senger <i>et al.</i> , "Tumor Cells Secrete a Vascular Permeability Factor That Promotes Accumulation of Ascites Fluid," <i>Science</i> , 219:983-985 (February 25, 1983).
	C91	Shalaby <i>et al.</i> , "Failure of Blood-Island Formation and Vasculogenesis in Flk-1-deficient Mice," <i>Nature</i> , 376:62-66 (July 6, 1995).
	C92	Shibuya <i>et al.</i> , "Nucleotide Sequence and Expression of a Novel Human Receptor-Type Tyrosine Kinase Gene (flt) Closely Related to the fms Family," <i>Oncogene</i> , 5:519-524 (1990).
	C93	Shibuya, M., "Role of VEGF-FLT Receptor System in Normal and Tumor Angiogenesis," <i>Adv. Cancer Res.</i> , 67:281-316 (1995).
	C94	Shweiki <i>et al.</i> , "Patterns of Expression of Vascular Endothelial Growth Factor (VEGF) and VEGF Receptors in Mice Suggest a Role in Hormonally Regulated Angiogenesis," <i>J. Clin. Invest.</i> , 91:2235-2243 (May, 1993).
	C95	Sitaras <i>et al.</i> , "Constitutive Production of Platelet-Derived Growth Factor-Like Proteins by Human Prostate Carcinoma Cell Lines," <i>Cancer Research</i> , 48(7):1930-1935 (April 1, 1988).
	C96	Southern and Berg, "Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter," <i>J. Mol. Appl. Genet.</i> , 1:327-341 (1982).
	C97	Terman <i>et al.</i> , "Identification of New Endothelial Cell Growth Factor Receptor Tyrosine Kinase," <i>Oncogene</i> , 6:1677-1683 (1991).
	C98	Terman <i>et al.</i> , "Identification of the KDR Tyrosine Kinase as a Receptor for Vascular Endothelial Cell Growth Factor," <i>Biochem. Biophys. Res. Commun.</i> , 187:1579-1586 (September 30, 1992).
	C99	Terman <i>et al.</i> , "VEGF Receptor Subtypes KDR and FLT1 Show Different Sensitivities to Heparin and Placenta Growth Factor," <i>Growth Factors</i> , 11(3):187-195 (1994).
	C100	Tessier <i>et al.</i> , "Enhanced Secretion From Insect Cells of a Foreign Protein Fused to the Honeybee Melittin Signal Peptide," <i>Gene</i> , 98: 177-183 (1991).
	C101	Tischer <i>et al.</i> , "The Human Gene for Vascular Endothelial Growth Factor. Multiple Protein Forms are Encoded Through Alternative Exon Splicing," <i>J. Biol. Chem.</i> , 266(18):11947-11954 (June 25, 1991).

EXAMINER Brian Lathrop	DATE CONSIDERED 5/8/97
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28113/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 01/12/96	Group 1806 1801

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
<i>B11</i>	C102	Van der Geer <i>et al.</i> , "Receptor Protein-Tyrosine Kinases and Their Signal Transduction Pathways," <i>Ann. Rev. Cell Biol.</i> , 10:251-337 (1994).
	C103	Vassar <i>et al.</i> , "Tissue-specific and Differentiation -specific Expression of a Human K14 Keratin Gene in Transgenic Mice," <i>Proc. Nat'l Acad. Sci., USA</i> , 86:1563-1567 (March, 1989).
	C104	Vassar <i>et al.</i> , "Transgenic Mice Provide New Insights Into the Role of TGF- α During Epidermal Development and Differentiation," <i>Genes & Dev.</i> , 5:714-727 (1991).
	C105	Västrik <i>et al.</i> , "Expression of the <i>Mad</i> Gene During Cell Differentiation <i>In Vivo</i> and Its Inhibition of Cell Growth <i>In Vitro</i> ," <i>J. Cell. Biol.</i> , 128(6):1197-1208 (March, 1995).
	C106	von Heijne, G., "A New Method for Predicting Signal Sequence Cleavage Sites," <i>Nucleic Acids Res.</i> , 14(11):4683-4690 (1986).
	C107	Waltenberger <i>et al.</i> , "Different Signal Transduction Properties of KDR and Flt1, Two Receptors for Vascular Endothelial Growth Factor," <i>J. Biol. Chem.</i> , 269(43):26988-26995 (October 28, 1994).
	C108	Wanaka <i>et al.</i> , "Expression of FGF Receptor Gene in Rat Development," <i>Development</i> , 111:455-468 (1991).
	C109	Wen <i>et al.</i> , "Neu Differentiation Factor: A Transmembrane Glycoprotein Containing an EGF Domain and an Immunoglobulin Homology Unit," <i>Cell</i> 69:559-572 (May 1, 1992).
	C110	Yamane <i>et al.</i> , "A New Communication System Between Hepatocytes and Sinusoidal Endothelial Cells in Liver Through Vascular Endothelial Growth Factor and Flt Tyrosine Kinase Receptor Family (Flt-1 and KDR/Flk-1)," <i>Oncogene</i> , 9:2683-2690 (1994).

EXAMINER <i>Brian Lathrop</i>	DATE CONSIDERED <i>5/8/97</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28113/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT <i>(Use several sheets if necessary)</i>		Applicant Alitalo et al.	
		Filing Date 01/12/96	Group 1806 1801

U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate

FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No
BW	B2	WO 96/30046	10/03/96	PET WJ	—		
BW	B3	WO 95/33050	12/07/95	PET WO	—		

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

EXAMINER Brian Lathrop	DATE CONSIDERED 5/8/97
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance <u>and</u> not considered. Include copy of this form with next communication to applicant.	

SHEET 1 of 1

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28113/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo et al.	
		Filing Date 01/12/96	Group 1806 1821

U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing I. If Appropriat

FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No
Full	B5	WO 96/39421	12/12/96	PCT-WO	—	—	—
Full	B6	WO 96/39515	12/12/96	PCT-WO	—	—	—

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

EXAMINER Brian Lathrop	DATE CONSIDERED 5/8/97
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	



#171
ms
01/16/97

PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No. 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

) I hereby certify that this paper is
) being deposited with the United
) States Postal Service as first class
) mail, postage prepaid, in an
) envelope addressed to: Assistant
) Commissioner for Patents,
) Washington, D.C. 20231, on this
) date:

) Dated: November 26, 1997

) 
) David A. Gass

AMENDMENT AND REPLY PURSUANT TO
37 C.F.R. §§ 1.111 AND 1.115

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In an official action mailed May 28, 1997, the U.S. Patent and Trademark Office (the Patent Office) rejected claims 1, 3-7, 11, 13, and 17-25 variously under 35 U.S.C. §§ 112, first and second paragraphs. The Applicants respectfully request reconsideration in light of the following amendments and remarks. This Amendment and Reply has been timely filed with a petition and fee for three months extension of time, extending the time period for response until November 28, 1997.

AMENDMENTS

In the specification:

Please amend the specification as follows:

At page 4, lines 14 and 21, delete "Flt-4" and substitute therefor --

Flt4 --

At page 5, line 14, delete "32" and substitute -- 33 --.

At page 5, line 17, delete "318" and substitute -- 317 --.

At page 6, line 31, delete "97321" and substitute therefor --

97231 --

At page 8, line 14, delete "Figure 5 shows" and substitute therefor

-- Figures 5A, 5B, and 5C show --.

At page 8, lines 19-20, delete "fractions from the Western analysis" and substitute therefor -- chromatographic fractions from the affinity purification --.

At page 8, please delete the brief description of Figure 10 at lines 30-32, and substitute therefor:

--
Figures 10A-10B show a comparison of the deduced amino acid sequences of PDGF-A (SEQ ID NO: 36); PDGF-B (SEQ ID NO: 37); two PIGF isoforms (SEQ ID NOs: 38 and 39); four VEGF isoforms (SEQ ID NOs: 40-43); and Flt4 ligand (VEGF-C) (SEQ ID NO: 33).--.

At page 9, line 5, after "lines" please insert -- and in brain tissue --.

At page 9, lines 6 and 10, delete "VEFG-C" and substitute therefor -- VEGF-C --.

At page 9, line 27, delete "its cloning" and substitute therefor -- the cloning of a DNA encoding this growth factor --.

At page 10, lines 1-2, delete "Claimed ligands" and substitute therefor -- Ligands of the invention --.

At page 11, line 7, after "residues" insert -- of --.

At page 11, line 19, delete "Balbaini" and substitute therefor -- Balbiani --.

At page 12, line 2, delete "have" and substitute therefor -- has --.

At page 12, line 7, delete "to structure to" and substitute therefor -- in structure to --.

At page 12, line 23, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 12, line 25, after "cells" insert -- (BCE) --.

At page 13, line 14, delete "the".

At page 13, line 17, delete "diseases" and substitute therefor -- diseases --.

At page 13, line 17, delete "to".

At page 14, line 4, delete "genes" and substitute therefor -- gene --.

At page 14, line 15, delete "these genes" and substitute therefor -- this gene --.

At page 14, line 20, delete "have" and substitute therefor -- has --.

At page 15, line 6, delete "the".

At page 15, line 12, after "Centricon 100" insert -- filters --.

At page 17, line 3, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 17, lines 9 and 13, delete "analysed" and, in each instance, substitute therefor -- analyzed --.

At page 17, line 13, after "50 μ g" insert -- of --.

At page 17, line 13, delete "was" and substitute therefor -- were --.

At page 17, line 18, delete "carboxyterminal" and substitute therefor -- carboxy-terminal --.

At page 19, lines 26 and 28, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 20, line 15, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 20, line 30, delete "was" and substitute therefor -- were --

At page 21, line 16, delete "3" and substitute therefor -- 4 --

At page 21, line 27, delete "extracellular" and substitute therefor -- extracellular --

At page 22, line 5, delete "dialysed" and substitute therefor -- dialyzed --

At page 22, line 14, delete "NIH3T3" and substitute therefor -- NIH 3T3 --

At page 23, line 4, delete "Malborough" and substitute therefor -- Marlborough --

At page 23, line 19, delete "was" and substitute therefor -- were --

At page 24, line 21, delete "analysed" and substitute therefor -- analyzed --

At page 25, line 29, delete "analysed" and substitute therefor -- analyzed --

At page 27, line 17, delete "analysed" and substitute therefor -- analyzed --

At page 27, line 33, delete "32" and substitute therefor -- 33 --

At page 28, line 17, delete "NIH3T3" and substitute therefor -- NIH 3T3 --

At page 28, line 19, delete "analysed" and substitute therefor -- analyzed --

At page 28, line 26, delete "slur" and substitute therefor -- slurry --

At page 29, line 1, after "97231.", please insert -- A 1997 base pair nucleotide sequence of the cDNA insert of this deposited plasmid is set forth in SEQ ID NOs: 44 and 45. --

At page 29, line 11, delete "two" and substitute therefor -- three --

At page 29, line 17, delete "Balbiani ring protein 3 (BRP3)" and substitute therefor -- Balbiani ring 3 protein (BR3P) --.

At page 29, line 22, delete "BRP3" and substitute therefor -- BR3P --.

At page 30, line 9, delete "assayed" and substitute therefor -- assayed --.

At page 30, line 11, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 30, line 34, delete "be" --.

At page 31, line 4, delete "analysed" and substitute therefor -- analyzed --.

At page 31, line 7, delete "10⁹" and substitute therefor -- 10⁹ --.

At page 31, line 23, delete "Metabolical" and substitute therefor -- metabolic --.

At page 31, line 30, delete "30 ml of a slur" and substitute therefor -- 30 μ l of a slurry --.

At page 32, line 3, delete "receptor binding" and substitute therefor -- receptor-binding --.

At page 32, line 24, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 32, line 34, delete "ml" and substitute therefor -- μ l --.

At page 33, line 3, delete "TBS" and substitute therefor -- RIPA --.

At page 33, lines 6, 14, and 25, delete "analysed" and, in each instance, substitute therefor -- analyzed --.

At page 33, line 19, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 33, line 31, after "(" please insert -- Fig. 14A --.

At page 34, line 10, delete "analysed" and substitute therefor -- analyzed --.

At page 34, line 14, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 36, line 20, delete "highly expressed in all tissues analysed" and substitute therefor -- highly expressed in all tissues analyzed --.

At page 37, line 19, delete "Gaithersburg" and substitute therefor -- Gaithersburg --.

At page 38, line 3, delete "analysed" and substitute therefor -- analyzed --.

At page 38, line 12, delete "VEGF-B" and substitute therefor -- VEGF-C --.

At page 38, line 27, delete "c6" and substitute therefor -- C6 --.

At page 39, line 16, delete "20952" and substitute therefor -- 20852 --.

Please delete pages 40-49 of the specification, which comprise the original sequence listing, and substitute therefor new pages 40-60 filed herewith, which constitute a substitute Sequence Listing. In view of this amendment, please renumber the pages of claims and abstract beginning with "61" (to preserve consecutive page numbering).

In the claims:

Please cancel claims 6, 13, and 17; amend claims 1, 3-5, 7, 11, 18, and 20; and add new claims 26-38 as shown below:

1. (Twice amended) A host cell transformed or transfected with a [purified and isolated] polynucleotide encoding a polypeptide [which specifically binds] that is capable of binding with high affinity to the extracellular domain of human Flt4 receptor tyrosine kinase,

wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32.
under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

C4
Cord: wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

Sink
D6
C5
3. (Twice amended) A host cell transformed or transfected with a [purified and isolated] nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

4. (Amended) A host cell [The nucleic acid] according to claim 3 wherein said nucleic acid comprises [having] the sequence shown in SEQ ID NO: 32.

5. (Twice amended) A host cell according to claim 3 wherein said polynucleotide is a vector comprising a nucleic acid that encodes a polypeptide having the amino acid sequence shown in SEQ ID NO: 33 [the nucleic acid according to claim 3].

Sub D7
C6

7. (Amended) A host cell comprising plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses a polypeptide encoded by said plasmid, said polypeptide including a domain defined by eight conserved cysteines having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P) [the vector according to claim 6].

C7

11. (Twice amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises [approximately] amino acids 1 to 120 of SEQ ID NO: 33.

Sub D8
C8

18. (Amended) A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33[, said portion encoding a polypeptide capable of binding to an Flt4 receptor tyrosine kinase] effective to permit such binding, said polynucleotide lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

C9

20. (Amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide has an apparent molecular weight of about 23 [kd] kD as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

C10
Sub D10

-- 26. A host cell according to claim 1 that expresses a naturally occurring VEGF-C protein encoded by said polynucleotide.

27. A host cell according to claim 1 that expresses a human VEGF-C protein encoded by said polynucleotide.

28. A host cell according to claim 27, wherein said host cell expresses said polynucleotide and produces a mature human VEGF-C protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to a nucleotide sequence that encodes said polypeptide.

30. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes a VEGF-homologous portion of SEQ ID NO: 33 and excludes the portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

C¹⁰
cont.
31. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide consisting of a continuous portion of the sequence shown in SEQ ID NO: 33, said continuous portion commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

34. An expression construct comprising the polynucleotide according to claim 33 operatively linked to an expression control sequence.

35. A host cell transformed or transfected with the expression construct of claim 34.

36. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

37. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight

conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

C¹⁰
word.

38. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 25 under conditions which permit expression by said host cell of a polypeptide encoded by said nucleic acid that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase; and

isolating said polypeptide from the host cell or the growth medium of the host cell. --

REMARKS

I. History of claims and explanation of amendments.

A. Prosecution History

The application as filed contained 16 claims.

In an official communication dated November 25, 1996, claims 1-16 were subjected to a restriction requirement. In an Amendment and Election in Response to Restriction Requirement filed on January 24, 1997, the Applicants: elected claims directed to nucleic acids, vectors, and host cells; canceled claims 2, 8-10, 12, and 14-16; amended claims 1, 3, 5, 11, and 13; and added claims 17-25.

In the outstanding Office action dated May 28, 1997, claims 1-3, 7, 11, 13, 17-25 were rejected. In the present amendment, the Applicants cancel claims 6, 13, and 17; amend claims 1, 3-5, 7, 11, 18, and 20; and add new claims 26-38. Thus, claims 1, 3-5, 7, 11 and 18-38 are now pending. A copy of the claims, in their amended forms, is appended hereto for the Examiner's convenience.

The Applicants do not intend by the amendments herein or any other amendments to abandon the subject matter of any claim as originally filed or as previously amended, and reserve the right to pursue such subject matter in subsequent applications, such as continuations, continuations-in-part, and divisional applications.

B. Amendments to the specification.

Most of the amendments to the specification correct obvious typographical errors, grammatical errors, and the like.

The amendments at page 5, lines 14 and 17, correct obvious typographical errors in the designation of the portions of SEQ ID NO: 33 which correspond to the unprocessed and "mature" forms of VEGF-C. The description of the amino terminus of a mature form of VEGF-C is found in the specification at, e.g., p. 23, lines 5-10, and is confirmed at page 25, line 27, to page 26,

line 6 (from which it is apparent that the first 13 amino acid residues of a secreted Flt4 ligand are encoded by the thirty-nine 3' bases of SEQ NO: 25 that begin ACAGAAGAGACT...). From these excerpts of the specification that identify the amino terminus of a mature VEGF-C protein, it is clear that the residues of SEQ ID NO: 33 as originally filed were misnumbered. As explained in the accompanying statement, a corrected SEQ ID NO: 33 has been filed herewith.

The amendment at page 6 to correct the ATCC accession number corrects an obvious typographical error, as is apparent from the ATCC deposit information provided at page 39, lines 14-18.

The amendment to the description of Figure 7 at page 8, lines 19-20; finds support at pages 21-22 (Example 5), from which it is apparent that Figure 7 depicts the results of gel electrophoresis of chromatographic fractions from the affinity purification of the Flt4 ligand.

The description for Figure 10 has been amended to reflect that Figure 10 is presently two panels (Figs. 10A-10B) and to include cross-references to the amended sequence listing. These amendments are responsive to objections raised in paragraphs 5 and 6 of the Office action.

The amendment at page 9, line 5, to add "brain tissue" to the description of Figure 11 finds support in Figure 11 itself, wherein the gel lane depicting the results for brain tissue is clearly labeled.

The amendments at pages 11 and 29 to correctly designate "Balbiani ring 3 protein" find support in the articles referenced at page 11, and the correct designations would have been understood by one skilled in the art.

The corrected cross-reference to Example 4 at page 21, line 16, finds support in Example 4 itself, because it is readily apparent from reading the application that Example 4 characterizes the ligand expressed by PC-3 cells.

The amendment at page 27, line 33, finds support as described above for the similar amendment at page 5, line 14.

The amendments to add SEQ ID NOs: 44-45 and include a cross-reference thereto at page 29, line 1, find support in the deposited plasmid

pFLT4-L, as an inherent property of the plasmid. See *In re Lundak*, 227 U.S.P.Q. 90 (Fed. Cir. 1985); *Therma-Tru Corp v. Peachtree Doors Inc.*, 33 U.S.P.Q.2d 1274, 1276 (Fed. Cir. 1995) ("[T]he later explicit description of an inherent property does not deprive the product of the benefit of the filing date of the earlier application."); and *Kennecott Corp. v. Kyocera International Inc.*, 5 U.S.P.Q.2d 1194 (Fed. Cir. 1987) (The express description of an inherent property is not new matter and can be added to a specification with effect as of the original filing date).

The amendment at page 29, line 11 finds support in Figure 10, wherein three (not two) putative N-linked glycosylation sites are underlined.

The substitution of " μ l" for "ml" at page 32, line 33, would have been obvious to the person of ordinary skill in the art from the context of Example 14, due to the nature of the experiment.

The corrected designation of a wash buffer used in the VEGFR-2 binding experiments at page 33, line 3 (Example 14), improves the readability of the eventual patent. This correction does not relate to how one would make or use the subject matter of the claims.

The amendment at page 33, line 31, to provide a cross-reference to Fig. 14A finds support in the context of the discussion of Figs. 14A-14B at page 33 and in the figures themselves, and will improve the readability of the eventual patent.

Support for the amendment at page 38, line 12, to substitute "VEGF-C" for "VEGF-B" is apparent from the context at page 38, lines 10-18, from which it is apparent that VEGF-C-encoding DNA is being discussed.

Support for the substitute Sequence Listing filed herewith is provided in the accompanying statement filed herewith.

C. Amendments to the claims.

All of the claim amendments find support throughout the application as originally filed.

For example, the recitations in claim 1 relating to binding to the extracellular domain of human Flt4 find support at p. 5, lines 4-9; p. 9, lines 30-32; p. 10, lines 26-31; p. 14, lines 30-34; and Examples 4 and 5.

The hybridization conditions recited in claim 1 find support in Example 10 (especially at p. 27, lines 9-14), wherein the recited hybridization conditions were employed in the isolation of VEGF-C-encoding cDNAs from a cDNA library.

The recitations in claims 1, 3, 7, 18, and 30 regarding expression of a polypeptide that includes a VEGF-homologous domain but excludes any domain having homology to a Balbiani ring 3 protein finds support in Example 13 (teaching that transfected host cells express and secrete 32 kD and 23 kD forms of VEGF-C that bind Flt4); at p. 11, lines 11-23 (teaching that the 23 kD polypeptide is likely to represent the VEGF-homologous domain, and that the carboxy-terminal amino acid sequences that show a cysteine pattern reminiscent of the Balbiani ring 3 protein is at least partially cleaved off); at page 11, lines 33-35, and in Fig. 10 (describing and depicting the eight conserved cysteine residues of the PDGF/PIGF/VEGF family of proteins).¹

The recitation of plasmid pFLT4-L in claim 7 finds support in claim 6 (from which claim 7 originally depended).

The amendment of claim 20 to substitute "kD" for "kd" is not intended as a substantive change, but merely is intended to increase uniformity of the eventual patent.

The recitation of an expression vector in claim 29 finds support in Example 11, e.g., at p. 28, lines 5-13; and at p. 6, lines 27-31.

The amino acid ranges recited in claims 31 and 33 find support at page 5, lines 27-33.

¹ In Fig. 10, the eight conserved cysteines are readily apparent at positions 103, 130, 136, 139, 140, 147, 184, and 186. In SEQ ID NO: 33 of the amended sequence listing, these eight cysteines correspond to residues 29, 54, 60, 63-64, 71, 107, and 109.

Support for claims 36-38, directed to a method for producing a polypeptide with host cells of the invention, is found at p. 6, lines 32-35, for example.

II. The second inventors' declaration, filed August 12, 1996, is not defective.

In paragraph 2 of the Office action, the Patent Office alleged that the inventors' declaration was defective due to non-initialed alterations and failure to acknowledge that the application is a CIP. The alleged defects are rendered moot by the second inventors' declaration that accompanied the Applicants' preliminary amendment dated August 12, 1996. Copies of the amendment and declaration are attached hereto as Exhibits 1 and 2.

III. Proposed drawing correction.

In paragraphs 3-4 and 6 of the Office action, the Patent Office requested the submission of a proposed drawing correction and the amendment of the brief description of the drawing to identify the two pages of Figure 10 as "10A and "10B". Attached hereto as Exhibit 3 is a proposed (informal) drawing correction. The Brief description of the drawing has been appropriately amended as well, at page 8, line 32. Accordingly, these objections may now be withdrawn. The Applicants wish to defer formal correction of the drawings and submission of a petition for photograph drawings until the application is allowed.

IV. The Application is in compliance with the Sequence Rules.

In paragraph 5 of the Office action and in a Notice to Comply, the Patent Office requested that the Application be amended to include the Figure 10 sequences therein, and to include cross-references to the Sequence Listing in the brief descriptions of Figs. 9B and 10. The Application has been so amended. The sequence listing amendment is accompanied by an appropriate

statement confirming that no new matter has been introduced into the application. Accordingly, this objection may properly be withdrawn.

V. The Applicants request deferral of the requirement for a Budapest Treaty declaration.

In paragraph 7 of the Office action, the Patent Office rejected claims 6 and 7 under 35 U.S.C. § 112, first paragraph, alleging that access to biological deposit material was required to use the claimed invention. As indicated in the specification at p. 39, the biological deposit was made pursuant to the provisions of the Budapest Treaty. A statement confirming the availability of the deposit is filed herewith, rendering this rejection moot.

The Budapest Treaty declaration filed herewith is intended solely to expedite prosecution, and is not intended as an admission that the deposited plasmid is required to satisfy § 112, first paragraph.

VI. The rejection of claim 1 under § 112, first paragraph, should be withdrawn.

In paragraph 8 of the Office action, the Patent Office rejected claim 1, alleging that the full scope of the claim was not enabled by the specification:

Claim 1 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for polynucleotides [sic: encoding?] all polypeptides that bind the Flt4 receptor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

The scope of claim 1 encompasses polynucleotides from any source that encode polypeptides that bind specifically to the Flt4 receptor. Making the invention requires testing all tissues from all known species, because neither the source nor the structure of the encoded proteins

are recited in the instant claims. There is no guidance provided by the specification to select those encompassed polynucleotides that encode proteins that specifically bind the Flt4 receptor with the exception of those teachings which support the subject matter indicated as enabled. There is no guidance to predict *a priori* whether any protein would bind the receptor *without some information on the structure of the protein*, and this information was simply not available for all the proteins encompassed by the claims at the time of the invention.

(Office action at p. 5.)

The Applicants traverse-in-part and amend-in-part.

A. The amendments to claim 1 overcome the factual bases for the Patent Office's rejection.

Claim 1 has been amended such that it no longer encompasses every polynucleotide that encodes all polypeptides that bind the Flt4 receptor. The Applicants have adopted the Examiner's suggestion to include additional limitations relating to the structure of the encoded protein.

In particular, amended claim 1 is directed only to polynucleotides that encode polypeptides that: (1) have a VEGF-homologous domain defined by eight conserved cysteines that are common to the PIGF/PDGF/VEGF family of polypeptides (see Fig. 10, positions 103, 130, 136, 139, 140, 147, 184, and 186); and (2) are capable of binding with *high affinity* to the *extracellular domain* of *human* Flt4. Thus, claim 1 has been further limited with respect to the source (human) and the domain (extracellular) of the binding partner; the nature (high affinity) of the binding reaction; and the type of polypeptide encoded (polypeptides which possess homology to a core portion of VEGF that is definable by eight conserved cysteines).

In addition, claim 1 now contains a significant structural limitation relating to the sequence of the claimed polynucleotide, namely, that the polynucleotide is sufficiently similar to the exemplified SEQ ID NO: 32 such that it will hybridize to the non-coding strand complementary to SEQ ID NO: 32 under specified hybridization conditions. The specified hybridization conditions

are those that were successfully employed in Example 10 to screen a PC-3 cell cDNA library to clone VEGF-C cDNA. (See specification at pp. 26-27.)

The scope of amended claim 1 is commensurate with the teachings in the application. Because of the hybridizing limitation, claim 1 reads only on those polynucleotides that can be identified via a routine hybridization screening assay that has been taught and successfully performed in the present application. Moreover, there is guidance in the specification that Flt4 ligands of the invention contain homology to VEGF (see, e.g., specification at p. 11, lines 11-13, and Figs. 10A-10B), and claim 1 has been appropriately limited in this manner. The specification teaches Flt4 binding assays that are useful to determine whether an encoded polypeptide binds Flt4.

In fact, subsequent hybridization experiments, using the human VEGF-C cDNA as a probe, were successfully performed to isolate VEGF-C-encoding cDNAs of mouse and quail. (See Declaration Under 37 C.F.R. §1.132 of Dr. Kari Alitalo at ¶¶ 10-18.) The identify of the encoded proteins was confirmed by receptor binding and stimulation studies. (*Id.*) This evidence that the specification enables one to isolate non-human VEGF-C-encoding cDNAs and confirm their identity in the receptor binding studies refutes the basis for the Patent Office's rejection.

For all of these reasons, claim 1 as amended is commensurate in scope with the teachings in the application, and the rejection under §112, first paragraph, should be withdrawn.

B. The Borg reference cited by the Patent Office does not support the rejection.

The Patent Office also cited a Borg publication in support of its rejection:

There is no guidance provided by the state of the art to select ligands to make the invention; despite intense research in this area, Borg et al. (reference C7) teach that n known ligands for the Flt4 receptor were known at the time of the invention.

(Office action at pp. 5-6.)

Reliance upon Borg is misplaced, because the Applicants do not rely upon Borg to provide an enabling disclosure. The Applicants specification provides the enabling disclosure. As explained above, amended claim 1 is commensurate in scope with guidance provided in the specification.

- C. The legal authorities relied upon by the Patent Office do not support a rejection of claim 1.

The Patent Office cites several cases in support of its rejection of claim 1:

The amount of guidance required varies inversely with the degree of predictability involved, and in applications directed to inventions in arts where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims. MPEP 2164.03 citing *In re Soll*, 97 F.2d 623, 38 USPQ 189 (CCPA 1938) and *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970). See also *Genentech, Inc. v. Novo Nordisk A/S*, 42 USPQ2d 1001 (Fed. Cir. 1997). For the reasons set forth above, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

(Office action at p. 6.)

The *Soll* opinion relied upon by the Patent Office is distinguishable because the patent applicant in that case was attempting to claim more broadly than the original disclosure of the patent application, which disclosure gave no indication that the applicant regarded his invention as a generic one. See *In re Soll*, 38 U.S.P.Q. at 190. In the present case, claim 1 has been amended herein to claim more narrowly than the generic invention originally contemplated and claimed by the inventors.

The *Fisher* opinion relied upon by the Patent Office relates to patent applications filed in the 1949-1960 period, well in advance of the genetic engineering techniques that were available and known in the art at the time the present application was filed. See *In re Fisher*, 166 U.S.P.Q. at 19-20. Because the genetic engineering techniques known to those skilled in the art at the time of the present application drastically reduce experimentation relative to

the traditional techniques that were known at the time of *Fisher*, the *Fisher* opinion cannot properly be applied against the present case.

The Patent Office's reliance upon the *Genentech* decision also is misplaced, because the facts of that case are wholly dissimilar from the facts of the present case.

Initially, it should be observed that *Genentech* is a case wherein the Court analyzed whether a 1979 patent application satisfied the enabling disclosure requirement. See *Genentech Inc. v. Novo Nordisk A/S*, 42 U.S.P.Q.2d 1001, 1004 (Fed. Cir. 1997). The state of the art relating to recombinant DNA and proteins was in a state of relative infancy in 1979, as compared to the state of the art in the 1994-1996 time period during which the present series of applications were filed. Since the enabling disclosure requirement involves an analysis of whether an application enables one of ordinary skill in the art to make and use an invention, and since the skill in the art has advanced enormously since 1979, the *Genentech* opinion is of little relevance.

Moreover, *Genentech* involved a unique situation wherein a patentee re-filed a patent application with a wholly new claim, in an attempt to enjoin an alleged infringer. As characterized by the Federal Circuit, the unique factual circumstances were as follows: an unsolved problem in the art had been the difficulty of obtaining a human protein (hGH) from a precursor that contained added protein material; Genentech's specification taught a solution to the problem wherein hGH was expressed *without* the added material; yet Genentech was attempting, in its re-filed application, to "bootstrap" by claiming a wholly different solution to the problem, namely, expression via synthesis and processing of a cleavable fusion protein. *Genentech*, 42 U.S.P.Q.2d at 1005. Genentech's specification contained "no disclosure of any specific starting material or of any of the conditions under which [the claimed] process can be carried out." *Id.* In these circumstances, the Court found Genentech's patent invalid.

In contrast, the present application teaches the procedures necessary to identify polynucleotides according to claim 1. For example, the present application teaches hybridization assays to identify candidate polynucleotides from other cDNA libraries; expression techniques for expressing polypeptides; and screening techniques to identify those polypeptides that bind to the extracellular domain of human Flt4.

The final case relied upon by the Patent Office, *In re Wands*, actually *supports*, rather than negates, a conclusion of enablement. The *Wands* opinion stands for the proposition that "Enablement is not precluded by the necessity for some experimentation such as routine screening. . . . The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988). In *Wands*, the Federal Circuit reversed as improper a rejection under §112, first paragraph. The Court recognized that practitioners in the pertinent molecular biological art were prepared to perform multiple screening experiments of "negatives" in order to identify one "positive." *Wands*, 8 U.S.P.Q.2d at 1406. Moreover, for the purposes of evaluating whether experimentation is "undue," the Federal Circuit recognized that "an experiment" was not simply defined by the screening of a single clone, but rather, by a larger process that can involve producing and screening several clones:

Furthermore, in the monoclonal antibody art it appears that an "experiment" is not simply the screening of a single hybridoma, but is rather the entire attempt to make a monoclonal antibody against a particular antigen. This process entails immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics.

In re Wands, 8 U.S.P.Q.2d at 1407.

As explained more fully in Section VII, below, the present specification enables the present claims, under the standards established in *Wands*.

VII. The rejection of claims 18-25 under §112, first paragraph, should be withdrawn.

In paragraph 9 of the Office action, the Patent Office rejected claims 18-25, alleging that the full scope of the claims was not enabled by the specification:

Claims 18-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for the scope of polynucleotides commensurate with the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

(Office action at pp. 6-8.)

The Applicants traverse-in-part and amend-in-part.

A principal basis for the rejection relates to the alleged scope of encoded-polypeptides encompassed by the claims:

The breadth of claims 18-25 encompasses polynucleotides encoding all active fragments of the protein of SEQ ID NO:33, or those that are 23 kDa or 32 kDa in size. Claim 21 recites the limitation that the fragment comprises SEQ ID NO:13, but this claim encompasses polypeptides in which SEQ ID NO:13 is the only sequence derived from the protein of SEQ ID NO:33. The only guidance offered by the specification to chose fragments that may make the invention is provided at page 11 where at least residues 1-120 of SEQ ID NO:33 are taught to be required for activity by comparison to PDGF (*infra*).

(Office action at pp. 6-7.)

The Applicants respectfully submit that the breadth of the claims are commensurate in scope with the guidance in the specification.

- A. There is no undue experimentation involved in synthesizing the polynucleotides (or the encoded polypeptides) within the scope of the claims.**

By providing the amino acid sequence set forth in SEQ ID NO: 33, the specification enables one skilled in the art to make essentially any polypeptide comprising a portion of SEQ ID NO: 33, and enables one to make essentially any polynucleotide coding sequence for such polypeptide. For example, such polynucleotides may be synthesized using automated synthesizers or using recombinant techniques (e.g., using polynucleotides of the invention and/or variants thereof obtained by site-directed mutagenesis).

- B. There is no undue experimentation involved in screening polypeptides for the abilities to bind Flt4 or stimulate Flt4 phosphorylation.**

Claim 18 is limited to nucleic acids that comprise a sequence encoding a polypeptide comprising a portion of SEQ ID NO: 33 effective to permit binding to Flt4. This limitation is commensurate in scope with the teachings in the application, because the specification teaches that encoded polypeptides are Flt4 ligands, and teaches Flt4 binding assays (and phosphorylation assays) to determine whether a polypeptide is capable of binding to Flt4 receptor tyrosine kinase (and whether the peptide is capable of stimulating Flt4 autophosphorylation). (See, e.g., Examples 4-5.) Such assays are the "routine screening" type of assay contemplated by the Federal Circuit in the *Wands* opinion.

- C. The specification provides guidance for identifying portions of SEQ ID NO: 33 effective to permit Flt4 binding.**

The specification provides significant guidance for determining portions of SEQ ID NO: 33 that are effective to permit Flt4 binding. For example, although SEQ ID NO: 33 contains 350 amino acids, the specification provides guidance that the first 33 amino acids are not critical for Flt4 binding. (See, e.g., p. 23, lines 5-10, and p. 25, line 27, to p. 26, line 6, which teach that a mature form of VEGF-C lacks the first 33 residues of SEQ ID NO: 33.)

The specification further teaches that the amino acids essential for retaining Flt4 ligand activity are contained within approximately amino acids 1-120 of SEQ ID NO: 33, and that the proteolytic cleavage that produces a mature, naturally occurring Flt4 ligand occurs within approximately amino acids 1-180 of SEQ ID NO: 33. (Specification at p. 5, lines 27-31.) There is guidance that the observed ~23 kD polypeptide exemplified in the application is likely to represent the VEGF-homologous domain of VEGF-C, and that the carboxy-terminal sequences that contain cysteine motifs reminiscent of a Balbiani ring 3 protein are cleaved off. (Specification at p. 11, lines 4-23.) At page 11, lines 33-35, attention is drawn to the probable importance of eight conserved cysteine residues of VEGF-C, which correspond to residues 29, 54, 60, 63-64, 71, 107, and 109 of SEQ ID NO: 33. (See Figure 10 and the amended Sequence Listing filed herewith.)

Additionally, the specification outlines a protocol for defining that portion of SEQ ID NO: 33 which corresponds with the naturally-occurring Flt4 ligand. (See pp. 29-30.) Furthermore, the specification provides guidance to (a) generate progressive deletion products of the Flt4 ligand cDNA; (b) express these modified cDNAs; and (c) assay the resulting truncated protein forms, e.g., by studying their ability to induce Flt4 autophosphorylation. (Specification at p. 30, lines 6-17.) The Declaration Under 37 C.F.R. §1.132 of Dr. Kari Alitalo filed herewith provides evidence that such procedures were successful in further characterizing the natural processing of VEGF-C and in identifying VEGF-C fragments that are capable of binding Flt4. (See ¶¶ 6-9.)

Collectively, these teachings serve to both provide guidance for predicting the portions of SEQ ID NO: 33 that are effective to permit Flt4 binding; and (2) reduce the amount of experimentation required to determine the minimum portion of SEQ ID NO: 33 that is critical for receptor binding.

D. The Patent Office's reliance upon Heldin to support its rejection is improper.

The Patent Office cited a Heldin publication in support of its rejection:

Heldin et al. (reference C40), however, teach the criticality of structures throughout the corresponding region of PDGF, such as extended loop structures (Figure 2), disulfide bonds involving residue 1 (page 249, column 1), and specific residues including those up to residue 154 (*loc. cit.*). Moreover, comparison with PDGF is of limited predictive value, because the stereo-specific interaction required between VEGF-C and its receptor are different than those between PDGF and its receptor as evidenced by the fact that the two ligands do not bind the same receptors. Without additional structural information on the ligand, the skilled artisan cannot predict which additional fragments of the protein of SEQ ID NO:33 might bind the receptor. Recitation that the encoded proteins must be 23 or 32 kDa in size does not provide significant additional guidance or limitation to the scope of the claims, because this limitation does not exclude the presence of sequences unrelated to VEGF-C within the polypeptide, nor does it exclude various post-translational modifications known to profoundly influence the apparent molecular weight without affecting the primary sequence of the polypeptide. Where the art is unpredictable, as in the case of physiological activity, more guidance is required. In re Fisher, 166 USPQ 18 (CCPA 1970).

(Office action at p. 7.)

The Applicants agree that comparison with PDGF, *by itself*, would be of limited predictive value. However, as outlined above in Section C, comparison with PDGF is merely one of many factors taught in the specification for predicting which fragments bind the receptor; and the application teaches routine screening to determine which fragments *actually* bind the receptor.

- E. The Patent Office's suggestion that one skilled in the art must test every fragment of SEQ ID NO: 33 is incorrect.

Underlying the Patent Office's rejection is the implicit assumption that the experimentation involved to practice the invention would require the screening of every possible fragment of SEQ ID NO: 33:

The vast amount of experimentation required to test all the encompassed fragments is an additional factor to be considered in the overall determination of whether the experimentation required to make the invention is undue. For the reasons set forth, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

(Office action at pp. 7-8.)

As an initial matter, the Applicants wish to clarify that the claims as written are directed to polynucleotides comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to Flt4. The "testing" of polypeptide fragments for Flt4 binding determines whether polynucleotides encoding the fragments fall within the claims. Therefore, to the extent the claims have been interpreted as "encompassing" both binding and non-binding fragments, the Patent Office has improperly ignored a limitation of the claims.

Second, the Patent Office's assumption that one must test "all" fragments improperly ignores the significant guidance in the specification with respect to those portions of SEQ ID NO: 33 that are effective to permit binding to Flt4. This guidance drastically reduces the number of fragments that one would select for screening.

Moreover, the Patent Office's suggestion that it is necessary to test all fragments of SEQ ID NO: 33 ignores the scientific ability of one of ordinary skill in the art. Importantly, one of ordinary skill in the art would not conduct experimentation by haphazardly making all of the possible fragments of SEQ ID NO: 33 and testing their ability to bind the receptor. An artisan of ordinary skill understands that each fragment that is screened provides guidance as to that portion of SEQ ID NO: 33 that is effective for binding, and

that portion which is not.² An artisan of ordinary skill also understands techniques for accelerating a screening process,³ and techniques for screening multiple polypeptides *simultaneously*. Thus, the examiner's reasoning greatly overstates both the quantity and the nature of the experimentation required to practice the invention as claimed.

F. The basis for rejection is moot with respect to the Applicants new claims 30-36.

New claims 30-36 contain additional limitations (relative to rejected claims 18-25) that characterize portions of SEQ ID NO: 33 which are effective to permit Flt4 binding, and which are encoded by the claimed polynucleotide. These additional limitations render inapplicable the bases for rejection that the Patent Office alleged against claims 18-25. Evidence in support of the patentability of these claims is provided in paragraphs 6-9 of the Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo filed herewith.

VIII. The rejection of claims 3-5, 11, 13, and 17-25 under §112, second paragraph, should be withdrawn.

In paragraph 10 of the Office action, the Patent Office rejected claims 3-5, 11, 13, and 17-25 under 35 U.S.C. §112, second paragraph, alleging that these claims were indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicants regard as the invention.

² For example, a determination that a polypeptide comprising residues 1-120 of SEQ ID NO: 33 is effective to permit binding to Flt4 and that a polypeptide comprising residues 121-317 is ineffective to permit binding would provide significant guidance as to that portion of SEQ ID NO: 33 to further screen for effective fragments. Thus, the assertion that it would be necessary to screen "all" fragments of SEQ ID NO: 33 to practice the claimed invention relies upon the false assumption that individual screening assays will be performed without knowledge gained from prior screenings.

³ For example, it is within the skill of the art to synthesize spaced deletion mutants (e.g., residues 1-100, 1-110, 1-120, 1-130, 10-120, 20-120, etc.) from SEQ ID NO: 33, rather than successive deletion mutants (1-130, 1-129, 1-128, 1-127, 1-126 . . .), to more rapidly identify effective portions for binding Flt4.

The Patent Office relied upon a Human Genome Sciences publication in support of its rejection:

SEQ ID NO:32 encodes a protein whose amino terminus is indicated as residue 1, and the specification teaches that the amino terminal of a 23 kDa protein expressed from the polynucleotide has the amino terminus shown in SEQ ID NO:13; however, Human Genome Sciences, Inc. disclose DNA encoding a similar sequence (99% global identity using the Smith-Waterman algorithm with 100% identity to the instantly claimed protein in the amino terminus through the instant residue 8) whose amino terminus is indicated as residue -8 of the instant protein. It would have been understood in the art that a disclosure of a particular residue as "residue 1" would have meant that this residue was the amino terminus of the mature polypeptide; however, it was unclear which residue corresponds to the amino terminus of the encoded polypeptide, making the designation of a particular amino acid residue as a "residue 1" indefinite. Although Human Genome Sciences was published after the effective filing date, the publication is used to show that the instant claims were indefinite at the time of filing. MPEP 2124 citing *In re Glass*, 492 F.2d 1228, 1232 n. 6., 181 USPQ 31, 34 n.6 (CCPA 1974).

(Office action at p. 8.)

Clarification is in order.

- A. The present application teaches that amino acid 1 is the thre nine that is the 34th residue of SEQ ID NO: 33.

The Patent Office is correct that the specification teaches that th amino terminal amino acid of a 23 kDa protein expressed from a polynucleotide comprising SEQ ID NO: 32 has the amino terminus shown in SEQ ID NO: 13. This amino terminus corresponds with the 34th residue in SEQ ID NO: 33. As explained in the Statement Pursuant to 37 C.F.R. §1.825 filed herewith, the Sequence Listing has been amended herein to reflect the fact that this thre nine residue represents the amino terminus of a mature VEGF-C protein.

The amino terminus taught in the present application reflects the results of amino acid sequencing of a purified VEGF-C protein secreted from a human cell line. (See Specification at pp. 21-23 (ExampI 5).) Thus, the

Applicants' asserted amino terminus is based upon the scientific characterization of a secreted human protein.

- B. Human Genome Sciences did not establish the amino terminus of a mature VEGF-C protein, and the cited publication is a mere guess that Human Genome Sciences later withdrew.

Apparently, the Patent Office relies upon Human Genome Science's International Patent Publication WO 95/24473 (hereinafter "HGS1") in support of its rejection. As set forth below, the HGS1 publication contains no sound scientific data to render indefinite the present claims or to call into question the Applicants' determination of the correct amino terminus of a mature VEGF-C protein.

The HGS1 publication teaches a "VEGF2" polypeptide "comprising 350 amino acids residues of which *approximately* the first 24 amino acids represent the leader sequence." (HGS1 at p. 4 (emphasis added).) The HGS1 publication does not base its determination of a "mature" 326 amino acid polypeptide on any scientific data. In fact, the only purported expression studies in the HGS1 publication were *in vitro* expression of PCR-amplified portions of cDNAs. (HGS1 at pp. 28-29.) The *in vitro* expression machinery employed would not necessarily process the expressed protein, and the HGS authors do not even report any analysis of the amino terminus of this *in vitro* protein. Because the splice site in the HGS1 publication is pure speculation, the authors assert only that "the first 24 amino acids residues *are likely to be leader sequence* (HGS1 at p. 5 (emphasis added).)"⁴

In fact, International Patent Publication No. WO 96/39515 (hereinafter "HGS2"), a later publication by a different Human Genome Sciences authorship entity, seems to *refute* the definition of a "mature" amino terminus that is proffered in HGS1. More particularly, HGS2 alleges that "VEGF2 contains an open reading frame encoding a protein of 419 amino acid residues

⁴ Moreover, HGS1 fails to identify a VEGF2 binding partner and fails to demonstrate a VEGF2 biological activity.

of which approximately the first 23 amino acid residues are the putative leader sequence such that the mature protein comprises 396 amino acids...." (HGS2 at p. 7.) Thus, more than one year after HGS1, the Human Genome Sciences scientists have apparently retracted or contradicted the teachings in HGS1 relating to a proper definition of "mature" VEGF2. HGS2, like HGS1, contains no scientific study to define a true "mature" VEGF2.

Thus, a careful scientific analysis reveals that the present application properly defines an amino terminus for a mature VEGF-C protein, based on sequencing studies of a VEGF-C protein that is actually expressed in a human cell line. The multiple, different amino termini alleged by Human Genome Sciences in its publications are mere speculation, unsupported by scientific evidence. Because the Applicants' asserted amino terminus is scientifically supported and the Human Genome Sciences purported amino termini are mere speculation, the Patent Office's indefiniteness rejection of claims 3-5, 11, 13, and 17-25 should be withdrawn.

IX. The rejection of claims 3-5, 11, 13, and 17-25 under §112, second paragraph, should be withdrawn.

In paragraph 11 of the Office action, the Patent Office rejected claims 11 and 13, under 35 U.S.C. §112, second paragraph, alleging that these claims were indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention:

Specifically, the term "approximately" in claims 11 and 13 is a relative term which renders the claim indefinite. The term "approximately" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

(Office action at p. 9.)

Solely for the purpose of expediting allowance, the Applicants have amended claim 11 to remove the allegedly indefinite term. Claim 13 has been canceled

herein. These amendments render this rejection moot. The rejection should therefore be withdrawn.

X. Comments concerning the Examiner's statement of reasons for the indication of allowable subject matter.

In paragraph 13 of the Office action, the Examiner commented as follows about the state of the art:

The following is a statement of reasons for the indication of allowable subject matter: polynucleotides encoding the instantly claimed ligand appear to be novel over the prior art of record. Borg et al. (reference C7), for example, disclose that the ligand for Flt4 was not known in the art around the time of invention. Closest prior art is a DNA with about 99% identity to the claimed polynucleotide (Human Genome Sciences, Inc. reference B1), but the publication date antedates the effective filing date of the instant application. Other relevant prior art made of record below discloses a series of expressed sequence tags (ESTs) with high identity to large regions of the Flt4 ligand cDNA. The probable identity of these ESTs was not disclosed, and without the benefit of hindsight, the artisan at the time of invention would not have been motivated to use these ESTs to make the claimed invention. It was not known that these ESTs encoded a receptor ligand, nor was it known that these ESTs were nearly identical to the Flt4 ligand cDNA at the time of invention. In fact, the only EST posited to encode a particular protein was taught to encode a Balbiani ring protein (Hillier et al., EST-STS Accession No. T81690), hardly giving motivation to use the EST to find the claimed invention.

(Office action at pp. 9-10.)

The Applicants first wish to clarify that the publication date of reference B1 does *not* antedate the effective filing date of the instant application. It is apparent from the context of the Office action that this is what the Examiner intended.

Moreover, the Applicants respectfully submit that all of the claims in the present application would be patentable over reference B1, even if that reference (or a counterpart U.S. patent) constituted statutory prior art.

For example, claim 1 is directed to a host cell transformed or transfected with a polynucleotide of the invention, wherein the host cell expresses a polypeptide encoded by said polynucleotide, *said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)*. Reference B1 does not even suggest to try to recombinantly express a polypeptide lacking domains having BR3P cysteine motifs. Even if such a suggestion existed, there would be no reasonable expectation from reference B1 that such a polypeptide would bind Flt4. In fact, there is no recognition in reference B1 that any polypeptide binds Flt4. Thus, the subject matter of claim 1 is novel and unobvious over reference B1. It will be apparent that similar reasoning supports the novelty and nonobviousness of host cell claims 3, 4, 5, 7, 25-29, and 35, and of method claims 35-38.

Claim 18 is directed to a nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to Flt4, *said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said polynucleotide lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein*. It is totally unexpected from reference B1 that a nucleic acid that encodes a portion of SEQ ID NO: 33 that lacks the recited BR3P encoding sequences still encodes a polypeptide that is capable of binding Flt4. These unexpected properties support the unobviousness of claim 18 and claims that depend therefrom. Similar considerations support the unobviousness of claims 33-35 over reference B1.

Thus, all of the pending claims would remain patentable over reference B1, even if this reference were statutory prior art.

XI. Prosecution has been suspended in a related application.

Pursuant to 37 C.F.R. §1.56, the Applicants wish to apprise the Examiner that prosecution has been suspended in a parent application (U.S.S.N. 08/510,133) "because a reference relevant to the examination . . . may soon become available." As set forth in Section X, above, if the reference is a U.S. patent counterpart to reference B1, the reference should not prevent allowance of the present application. All of the pending claims are directed to subject matter that is patentably distinct from anything disclosed or suggested in reference B1.

CONCLUSION

For the foregoing reasons, the applicants respectfully request reconsideration, withdrawal of all claim rejections and objections to the specification, and allowance of claims 1, 3-5, 7, 11, and 18-38.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 

David A. Gass
Registration No. 38,153

Date: Nov 26, 1997

APPENDIX OF CLAIMS

1. (Twice amended) A host cell transformed or transfected with a polynucleotide encoding a polypeptide that is capable of binding with high affinity to the extracellular domain of human Flt4 receptor tyrosine kinase, wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

2. [CANCELED]

3. (Twice amended) A host cell transformed or transfected with a nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

4. (Amended) A host cell according to claim 3 wherein said nucleic acid comprises the sequence shown in SEQ ID NO: 32.

5. (Twice amended) A host cell according to claim 3 wherein said polynucleotide is a vector comprising a nucleic acid that encodes a polypeptide having the amino acid sequence shown in SEQ ID NO: 33.

6. [CANCELED]

7. (Amended) A host cell comprising plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses a polypeptide encoded by said plasmid, said polypeptide including a domain defined by eight conserved cysteines having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

8. [CANCELED]

9. [CANCELED]

10. [CANCELED]

11. (Twice amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises amino acids 1 to 120 of SEQ ID NO: 33.

12. [CANCELED]

13. [CANCELED]

14. [CANCELED]

15. [CANCELED]

16. [CANCELED]

17. [CANCELED]

18. (Amended) A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said polynucleotide lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

19. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide is capable of stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase.

20. (Amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide has an apparent molecular weight of about 23 kD as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

21. A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises an amino-terminal amino acid sequence set forth in SEQ ID NO: 13.

22. A purified and isolated nucleic acid according to claim 21 wherein said polypeptide comprises approximately 120 amino acids.

23. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

24. A vector comprising a nucleic acid according to claim 18.

25. A host cell transformed or transfected with a vector according to claim 24.

26. A host cell according to claim 1 that expresses a naturally occurring VEGF-C protein encoded by said polynucleotide.

27. A host cell according to claim 1 that expresses a human VEGF-C protein encoded by said polynucleotide.

28. A host cell according to claim 27, wherein said host cell expresses said polynucleotide and produces a mature human VEGF-C protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to a nucleotide sequence that encodes said polypeptide.

30. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes a VEGF-homologous portion of SEQ ID NO: 33 and excludes the portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

31. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide consisting of a continuous portion of the sequence shown in SEQ

ID NO: 33, said continuous portion commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

34. An expression construct comprising the polynucleotide according to claim 33 operatively linked to an expression control sequence.

35. A host cell transformed or transfected with the expression construct of claim 34.

36. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

37. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

38. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 25 under conditions which permit expression by said host cell of a polypeptide encoded by said nucleic acid that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Alitalo, Kari
Joukov, Vladimir
- (ii) TITLE OF INVENTION: RECEPTOR LIGAND
- (iii) NUMBER OF SEQUENCES: 45
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
(B) STREET: 6300 Sears Tower, 233 South Wacker Drive
(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/585,895
(B) FILING DATE: 12-JAN-1996
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/510,133
(B) FILING DATE: 01-AUG-1995
- (viii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/340,011
(B) FILING DATE: 14-NOV-1994
- (ix) ATTORNEY/AGENT INFORMATION:
(A) NAME: Gass, David A.
(B) REGISTRATION NUMBER: 38,153
(C) REFERENCE/DOCKET NUMBER: 28967/33072
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312/474-6300
(B) TELEFAX: 312/474-0448
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTCCTCGCT GTCCTGTCT

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCGCTGG
GACTCCTGGA

60

70

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACATGCATGC CCCGCCGGTC ATCC

24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGAATTCCC CATGACCCCA AC

22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT

33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATTTAGGTGA CACTATA

17

C3
GTX.

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT

34

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
1 5 10 15
Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
20 25 30
His Arg Gln Glu Ser Gly Phe Arg
35 40

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGAGTCGA CTTGGCGGAC T

21

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC

60

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs

C3
cont.

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC

34

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTTCGCTGTG ATGTGCACCA

20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCAGARGARA CNATHAA

17

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

C3
cont.

Glu Glu Thr Ile Lys
1 5

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAYTTNARD ATYTCNGT

18

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Thr Glu Ile Leu Lys
1 5

C3
ent.
(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTCGCTGCA GCACACTACA AC

22

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCNGTGTGT AGTGTGCTG

19

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Ala His Tyr Asn Thr Glu
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TAATACGACT CACTATAGGG

20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTGTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Phe Ala Ala Ala His Tyr Asn
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

C3
cont.

- (A) LENGTH: 219 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCACTATAGG GAGACCCAAG CTTGGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT 60
GTGGTGAAT TCGACGAACT CATGACTGTA CTCTACCCAG AATATTGGAA AATGTACAAG 120
TGTCAGCTAA GGCAAGGAGG CTGGCAACAT AACAGAGAAC AGGCCAACCT CAACTCAAGG 180
ACAGAAGAGA CTATAAAATT CGCTGCAGCA CACTACAAC 219

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACAGAGAACA GGCCAACC

18

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCTAGCATT AGGTGACAC

19

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AAGAGACTAT AAAATTCGCT GCAGC

25

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

C3
cont.

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCTCTAGAT GCATGCTCGA

20

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1140 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 37..1086

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 136..1086

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAACTC ATG ACT GTA CTC TAC CCA
Met Thr Val Leu Tyr Pro
-33 -30

54

GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA
Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln
-25 -20 -15

102

CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA
His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile
-10 -5 1 5

150

C3
ent.

AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp 10 15 20	198
AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp 25 30 35	246
GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro 40 45 50	294
TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu 55 60 65	342
CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu 70 75 80 85	390
ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe 90 95 100	438
GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg 105 110 115	486
CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln 120 125 130	534
TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn 135 140 145	582
CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp 150 155 160 165	630
GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC Ala Gly Asp Asp Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn 170 175 180	678
AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu 185 190 195	726
CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys 200 205 210	774
CAG TGT GTC TGT AAA AAC AAA CTC TTC CCC AGC CAA TGT GGG GCC AAC Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn 215 220 225	822
CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys 230 235 240 245	870
CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr 250 255 260	918
GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln 265 270 275	966

C3
cont.

ACA TGC AGC TGT TAC AGA CCG CCA TGT ACG AAC CGC CAG AAG GCT TGT	1014
Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys	
280 285 290	
GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA	1062
Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser	
295 300 305	
TAT TGG AAA AGA CCA CAA ATG AGC TAAGATTGTA CTGTTTCCA GTTCATCGAT	1116
Tyr Trp Lys Arg Pro Gln Met Ser	
310 315	
TTTCTATTAT GGAAACTGT GTTG	1140

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 350 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu
 -33 -30 -25 -20
 Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser
 -15 -10 -5
 Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu
 1 5 10 15
 Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro
 20 25 30
 Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn
 35 40 45
 Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys
 50 55 60
 Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu
 65 70 75
 Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys
 80 85 90 95
 Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser
 100 105 110
 Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu
 115 120 125
 Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr
 130 135 140
 Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp
 145 150 155
 Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
 160 165 170 175
 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys
 180 185 190

C3
 cont.

-50-

Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu
195 200 205
Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro
210 215 220
Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys
225 230 235
Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys
240 245 250 255
Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly
260 265 270
Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr
275 280 285
Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val
290 295 300
Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser
305 310 315

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TGAGTGAATTTGTAGCTGCTGTG

22

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TATTGCAGCAACCCCCACATCT

22

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 196 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

C3
ent.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Arg Thr Trp Ala Cys Leu Leu Leu Leu Gly Cys Gly Tyr Leu Ala
1 5 10 15
His Ala Leu Ala Glu Glu Ala Glu Ile Pro Arg Glu Leu Ile Glu Arg
20 25 30
Leu Ala Arg Ser Gln Ile His Ser Ile Arg Asp Leu Gln Arg Leu Leu
35 40 45
Glu Ile Asp Ser Val Gly Ala Glu Asp Ala Leu Glu Thr Ser Leu Arg
50 55 60
Ala His Gly Ser His Ala Ile Asn His Val Pro Glu Lys Arg Pro Val
65 70 75 80
Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Ile Pro Ala Val Cys
85 90 95
Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro
100 105 110
Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg
115 120 125
Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg
130 135 140
Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys
145 150 155 160
Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu
165 170 175
Cys Ala Cys Ala Thr Ser Asn Leu Asn Pro Asp His Arg Glu Glu Glu
180 185 190
Thr Asp Val Arg
195

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 241 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Met Asn Arg Cys Trp Ala Leu Phe Leu Ser Leu Cys Cys Tyr Leu Arg
1 5 10 15
Leu Val Ser Ala Glu Gly Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met
20 25 30
Leu Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu
35 40 45
His Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met
50 55 60

Thr Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Arg
65 70 75 80
Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu
85 90 95
Cys Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp
100 105 110
Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln
115 120 125
Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr
130 135 140
Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg
145 150 155 160
Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu
165 170 175
Ala Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr Arg Ser
180 185 190
Pro Gly Gly Ser Gln Glu Gln Arg Ala Lys Thr Pro Gln Thr Arg Val
195 200 205
Thr Ile Arg Thr Val Arg Val Arg Arg Pro Pro Lys Gly Lys His Arg
210 215 220
Lys Phe Lys His Thr His Asp Lys Thr Ala Leu Lys Glu Thr Leu Gly
225 230 235 240
Ala

C3
cont.

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 149 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1 5 10 15
Leu Ala Leu Pro Ala Val Pro Pro Gln Trp Ala Leu Ser Ala Gly
20 25 30
Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
35 40 45
Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
50 55 60
Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65 70 75 80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
85 90 95
Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
100 105 110
Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
115 120 125
Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Cys Gly Asp
130 135 140
Ala Val Pro Arg Arg
145

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1 5 10 15
Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
20 25 30
Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
35 40 45
Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
50 55 60
Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65 70 75 80
Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
85 90 95
Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
100 105 110
Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
115 120 125
Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Arg Arg Pro
130 135 140
Lys Gly Arg Gly Lys Arg Arg Arg Glu Lys Gln Arg Pro Thr Asp Cys
145 150 155 160
His Leu Cys Gly Asp Ala Val Pro Arg Arg
165 170

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Cys Asp Lys
130 135 140
Pro Arg Arg
145

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 191 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
130 135 140
Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145 150 155 160
Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
165 170 175
Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
180 185 190

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 215 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
130 135 140

C3
cont.

Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145 150 155 160
Lys Ser Trp Ser Val Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His
165 170 175
Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr
180 185 190
Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys
195 200 205
Arg Cys Asp Lys Pro Arg Arg
210 215

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
130 135 140
Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145 150 155 160
Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys Leu Met Pro Trp
165 170 175
Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys
180 185 190

C3
ent.

His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn
 195 200 205
 Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr
 210 215 220
 Cys Arg Cys Asp Lys Pro Arg Arg
 225 230

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1997 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CCCCCCCCGC CTCTCCAAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCCTCGCC 60
 CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCGGTTT CCTGTGAGGC 120
 TTTTACCTGA CACCCGCGCC CTTTCCCCGG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG 180
 GGAACGCGGA GCCCGGACCG CGCTCCCGCC GCCTCCGGCT CGCCAGGGG GGGTCGCGCG 240
 GAGGAGCCCC GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGGC GCCCGCGCCC 300
 CCACCCCTGC CCCCGCCAGC GGACCGGTCC CCCACCCCGG GTCCTTCAC C ATG CAC 357
 Met His
 1
 TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG 405
 Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Ala Leu
 5 10 15
 CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC GCC TTC GAG TCC 453
 Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser
 20 25 30
 GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT 501
 Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala
 35 40 45 50
 TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA 549
 Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val
 55 60 65
 GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG 597
 Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys
 70 75 80
 TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC 645
 Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn
 85 90 95

C3
 cont.

CTC	AAC	TCA	AGG	ACA	GAA	GAG	ACT	ATA	AAA	TTT	GCT	GCA	GCA	CAT	TAT	693
Leu	Asn	Ser	Arg	Thr	Glu	Glu	Thr	Ile	Lys	Phe	Ala	Ala	Ala	His	Tyr	
100						105					110					
AAT	ACA	GAG	ATC	TTG	AAA	AGT	ATT	GAT	AAT	GAG	TGG	AGA	AAG	ACT	CAA	741
Asn	Thr	Glu	Ile	Leu	Lys	Ser	Ile	Asp	Asn	Glu	Trp	Arg	Lys	Thr	Gln	
115					120					125					130	
TGC	ATG	CCA	CGG	GAG	GTG	TGT	ATA	GAT	GTG	GGG	AAG	GAG	TTT	GGA	GTC	789
Cys	Met	Pro	Arg	Val	Cys	Ile	Asp		Val	Gly	Lys	Glu	Phe	Gly	Val	
				135					140					145		
GCG	ACA	AAC	ACC	TTC	TTT	AAA	CCT	CCA	TGT	GTG	TCC	GTC	TAC	AGA	TGT	837
Ala	Thr	Asn	Thr	Phe	Phe	Lys	Pro	Pro	Cys	Val	Ser	Val	Tyr	Arg	Cys	
				150				155					160			
GGG	GGT	TGC	TGC	AAT	AGT	GAG	GGG	CTG	CAG	TGC	ATG	AAC	ACC	AGC	ACG	885
Gly	Gly	Cys	Cys	Asn	Ser	Glu	Gly	Leu	Gln	Cys	Met	Asn	Thr	Ser	Thr	
		165					170					175				
AGC	TAC	CTC	AGC	AAG	ACG	TTA	TTT	GAA	ATT	ACA	GTG	CCT	CTC	TCT	CAA	933
Ser	Tyr	Leu	Ser	Lys	Thr	Leu	Phe	Glu	Ile	Thr	Val	Pro	Leu	Ser	Gln	
	180					185					190					
GGC	CCC	AAA	CCA	GTA	ACA	ATC	AGT	TTT	GCC	AAT	CAC	ACT	TCC	TGC	CGA	981
Gly	Pro	Lys	Pro	Val	Thr	Ile	Ser	Phe	Ala	Asn	His	Thr	Ser	Cys	Arg	
195					200					205					210	
TGC	ATG	TCT	AAA	CTG	GAT	GTT	TAC	AGA	CAA	GTT	CAT	TCC	ATT	ATT	AGA	1029
Cys	Met	Ser	Lys	Leu	Asp	Val	Tyr	Arg	Gln	Val	His	Ser	Ile	Ile	Arg	
				215					220					225		
CGT	TCC	CTG	CCA	GCA	ACA	CTA	CCA	CAG	TGT	CAG	GCA	GCG	AAC	AAG	ACC	1077
Arg	Ser	Leu	Pro	Ala	Thr	Leu	Pro	Gln	Cys	Gln	Ala	Ala	Lys	Thr		
				230				235					240			
TGC	CCC	ACC	AAT	TAC	ATG	TGG	AAT	AAT	CAC	ATC	TGC	AGA	TGC	CTG	GCT	1125
Cys	Pro	Thr	Asn	Tyr	Met	Trp	Asn	Asn	His	Ile	Cys	Arg	Cys	Leu	Ala	
		245					250					255				
CAG	GAA	GAT	TTT	ATG	TTT	TCC	TCG	GAT	GCT	GGA	GAT	GAC	TCA	ACA	GAT	1173
Gln	Glu	Asp	Phe	Met	Phe	Ser	Ser	Asp	Ala	Gly	Asp	Asp	Ser	Thr	Asp	
	260				265					270						
GGA	TTC	CAT	GAC	ATC	TGT	GGA	CCA	AAC	AAG	GAG	CTG	GAT	GAA	GAG	ACC	1221
Gly	Phe	His	Asp	Ile	Cys	Gly	Pro	Asn	Lys	Glu	Leu	Asp	Glu	Glu	Thr	
275					280					285					290	
TGT	CAG	TGT	GTC	TGC	AGA	GCG	GGG	CTT	CGG	CCT	GCC	AGC	TGT	GGA	CCC	1269
Cys	Gln	Cys	Val	Cys	Arg	Ala	Gly	Leu	Arg	Pro	Ala	Ser	Cys	Gly	Pro	
				295					300					305		
CAC	AAA	GAA	CTA	GAC	AGA	AAC	TCA	TGC	CAG	TGT	GTC	TGT	AAA	AAC	AAA	1317
His	Lys	Glu	Leu	Asp	Arg	Asn	Ser	Cys	Gln	Cys	Val	Cys	Lys	Asn	Lys	
			310				315						320			
CTC	TTC	CCC	AGC	CAA	TGT	GGG	GCC	AAC	CGA	GAA	TTT	GAT	GAA	AAC	ACA	1365
Leu	Phe	Pro	Ser	Gln	Cys	Gly	Ala	Asn	Arg	Glu	Phe	Asp	Glu	Asn	Thr	
		325					330					335				
TGC	CAG	TGT	GTA	TGT	AAA	AGA	ACC	TGC	CCC	AGA	AAT	CAA	CCC	CTA	AAT	1413
Cys	Gln	Cys	Val	Cys	Lys	Arg	Thr	Cys	Pro	Arg	Asn	Gln	Pro	Leu	Asn	
	340				345						350					
CCT	GGA	AAA	TGT	GCC	TGT	GAA	TGT	ACA	GAA	AGT	CCA	CAG	AAA	TGC	TTG	1461
Pro	Gly	Lys	Cys	Ala	Cys	Glu	Cys	Thr	Glu	Ser	Pro	Gln	Lys	Cys	Leu	
355					360					365					370	

C 3
cont.



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):)	Title: RECEPTOR LIGAND
Alitalo et al.)	
Serial No: 08/585,895)	Group Art Unit: 1801
Filed: January 12, 1996)	Examiner: Lathrop, B.

**AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111 and 1.115, including:
(A) new pages 40-60 comprising a paper copy of a substitute Sequence Listing;
(B) Exhibits 1,2 and 3;
2. Computer-readable copy of substitute Sequence Listing;
3. Statement Pursuant to 37 C.F.R. 1.825(a) and 1.825(b);
4. Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo;
5. Declaration of Biological Culture Deposit in Compliance with Budapest Treaty Requirements;
6. Check in the amount of \$475.00 in payment of fee for extension of time; and
7. Check in the amount of \$360 in payment of fee for extra claims.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on November 26, 1997, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. Small Entity Status

- ☒ Small entity status has been established and is still effective.

2. Extension of Time

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month		\$110.00		\$55.00
Two Months		\$400.00		\$200.00
Three Months		\$950.00	X	\$475.00
Four Months		\$1,510.00		\$755.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$475.00

- ☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$_____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$0

Extension Fee Due With This Request \$475.00

3. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	33	MINUS	20	13	X11 =	\$143	X22 =	\$
INDEP.	5	MINUS	-3	2	X41 =	\$82	X82 =	\$
First Presentation of Multiple Dependent Claim					+135 =	\$135	+270 =	\$
TOTAL ADDITIONAL FEE						\$360	OR	\$

4. **Method of Payment of Fees**

Attached are checks in the amount of \$475 and \$360.

- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

5. **Deposit Account and Refund Authorization**

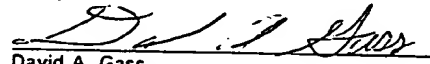
The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:


David A. Gass
Reg. No: 38,153

November 26, 1997



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):)	Title: RECEPTOR LIGAND
Alitalo et al.)	
Serial No: 08/585,895)	Group Art Unit: 1801
Filed: January 12, 1996)	Examiner: Lathrop, B.

AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111 and 1.115, including:
(A) new pages 40-60 comprising a paper copy of a substitute Sequence Listing;
(B) Exhibits 1,2 and 3;
2. Computer-readable copy of substitute Sequence Listing;
3. Statement Pursuant to 37 C.F.R. 1.825(a) and 1.825(b);
4. Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo;
5. Declaration of Biological Culture Deposit in Compliance with Budapest Treaty Requirements;
6. Check in the amount of \$475.00 in payment of fee for extension of time; and
7. Check in the amount of \$360 in payment of fee for extra claims.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on November 26, 1997, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed as Application Serial No. 08/585,895. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment attached hereto. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Priority Claimed
☐ Yes ☒ No

(Application Serial Number)	(Country)	(Day/Month/Year Filed)
050624	Finland	13 February 1995

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

(Application Serial Number)	(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurs between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)
08/340,011	14 November 1994	Pending
08/510,133	01 August 1995	Pending

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements are the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shelman (19,412)	Trevor B. Anke (25,542)	Richard A. Schmitt (30,890)	James J. Napoli (32,361)
Donald J. Brett (19,490)	Timothy J. Vezou (26,348)	Anthony Nimmo (30,920)	Richard M. La Barre (32,254)
Jean J. Murray (22,111)	Carl E. Moore, Jr. (26,487)	Christine A. Dudzik (31,245)	Jeffrey W. Smith (33,455)
Allen H. Gerstein (22,218)	Richard H. Anderson (26,526)	Kevin D. Hogg (31,839)	Douglas C. Huchmesler (33,710)
Nate F. Scarpelli (22,320)	Patrick D. Eriel (26,877)	Jeffrey S. Sharp (31,879)	Cynthia L. Schaller (34,245)
Edward M. O'Toole (22,477)	James P. Zeller (28,491)	Donald J. Pochopien (32,167)	Robert M. Gerstein (34,324)
Michael F. Borus (25,447)	William E. McCracken (30,195)	Martin J. Hirsch (32,237)	David A. Goss (38,153)

Send correspondence to: David A. Goss

FORM NAME	PHONE NO	STREET	CITY & STATE	ZIP CODE
Marshall, O'Toole, Gerstein, Murray & Borus	312 474-6300	6300 Sears Tower 233 South Wacker Drive	Chicago, Illinois	60606-6402

Full Name of First or Sole Inventor	Citizenship
Kari Alitalo	Finland
Residence Address - Street	Post Office Address - Street
Nyyrikintie 4A	Same
City (Zip)	City (Zip)
02100 Espoo	Same
State or Country	State or Country
FINLAND	Same
Date	Signature
June 6, 1996	[Signature]

See second page for additional inventor

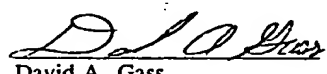
See reverse for relevant rules & statutes



Second Joint Inventor, if any Vladimir Joukov	Citizenship Russia
Residence Address - Street Topeliuksenkatu 32G8	Post Office Address - Street Same
City (Zip) 00290 Helsinki	City (Zip) Same
State or Country FINLAND	State or Country Same
Date ■ Aug. 6, 1996	Signature ■ <i>V. Joukov</i>

PATENT
28967/33072

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

In re Application of:)	I hereby certify that this paper is being
Alitalo et al.)	deposited with the United States Postal
Serial No.: 08/585,895)	Service as first class mail, postage
Filed: January 12, 1996)	prepaid, in an envelope addressed to:
Title: RECEPTOR LIGAND)	Assistant Commissioner for Patents
Art Unit: 1801)	Washington, D.C. 20231, on this date:
Examiner: Lathrop, B.)	Dated: <u>Nov. 26, 1997</u>
)	
)	David A. Gass
)	Registration No. 38,153

DECLARATION OF BIOLOGICAL CULTURE DEPOSIT
IN COMPLIANCE WITH BUDAPEST TREATY REQUIREMENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, the undersigned, declare that:

1. I am an inventor of the subject matter of the above-identified patent application.
2. The plasmid designated FLT4-L, described in the specification of the above-identified application at pages 28-29 (and elsewhere), was deposited on 24 July 1995 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, under the terms of the Budapest Treaty. This plasmid was assigned ATCC accession number 97231. A copy of the ATCC deposit receipt, confirming viability of the deposit, is attached hereto.

3. With respect to the permanence of the deposit, the ATCC is an official depository in accordance with the Budapest Treaty for the above-deposited material, and I affirm that, should the plasmid identified in paragraph 2 mutate, become non-viable, or be inadvertently destroyed, I will replace it for at least thirty (30) years from the date of the original deposit, or for at least five (5) years from the date of the most recent request for release of a sample, or for the enforceable life of any patent issued on the above-mentioned application, whichever period is longest.

4. With respect to availability of the plasmid identified in paragraph 2, I affirm that the deposit has been made under conditions of assurance of (a) ready accessibility thereto by the public if an enforceable patent is granted whereby all restrictions to the availability to the public of the culture so deposited will be irrevocably removed upon the granting of the patent [MPEP §608.01 (p)], and (b) access to the deposit will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122.

5. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

November 20, 1997
Date

Kari Alitalo
Kari Alitalo



American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301) 231-5520 Telex: 898-055 ATCCNORTH • FAX: 301-770-2587

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

University of Helsinki
Attention: Kari Alitalo
Molecular/Cancer Biology Laboratory
P.O. Box 21 (Haartmaninkatu 3)
SF-00014, HELSINKI, FINLAND

Deposited on Behalf of: Kari Alitalo and Vladimir Joukov

Identification Reference by Depositor:

ATCC Designation

Plasmid, FLT4-L

97231

The deposit was accompanied by: ☐ a scientific description ☐ a proposed taxonomic description indicated above.

The deposit was received July 24, 1995 by this International Depository Authority and has been accepted.

AT YOUR REQUEST:

☒ We will not inform you of requests for the strain.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested August 1, 1995. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:


Annette L. Bade, Director, Patent Depository

Date: August 9, 1995

cc: Thomas C. Meyers



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No. 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

) I hereby certify that this paper is
) being deposited with the United
) States Postal Service as first class
) mail, postage prepaid, in an
) envelope addressed to: Assistant
) Commissioner for Patents,
) Washington, D.C. 20231, on this
) date:

) Dated: November 26, 1997

) David A. Gass
)
) David A. Gass

STATEMENT PURSUANT TO 37 C.F.R. §1.825(a) and §1.825(b)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby state that the content of the paper and computer-readable forms of the substitute Sequence Listing submitted herewith, for entry as part of the above-identified application, are the same as each other and do not introduce new matter into the disclosure of the application. All of the amendments embodied in the substitute Sequence Listing filed herewith find support in the application as originally filed.

SEQ ID NOs: 1-31 and 34-35 of the original and substitute Sequence Listings are identical. Therefore, no new matter has been introduced in these sequences.

SEQ ID NOs: 36-43 have been added to the substitute Sequence Listing pursuant to instructions from the Patent Office to include sequences therein that are depicted in Figure 10 of the application. Because these eight sequences all find support in Figure 10 as originally filed, they do not introduce

new matter. Appropriate cross-references to SEQ ID NOs: 36-43 have been included in the brief description of the drawing.

SEQ ID NOs: 32-33 of the original and substitute Sequence Listings are identical. However, the amino acid numbering of these sequences has been amended in the substitute sequence listing by identifying the 34th residue in the substitute sequence listing as residue 1. (In the original sequence listing, the 33rd residue was identified as residue 1.) This amendment finds support throughout the specification as originally filed. For example, the description of the amino terminus of a mature form of VEGF-C is found in the specification at p. 23, lines 5-10, and is confirmed at page 25, line 27, to page 26, line 6 (from which it is apparent that the first 46 codons comprise 33 "signal sequence" residues and 13 amino acid residues of a secreted Flt4 ligand). From these excerpts of the specification that identify the amino terminus of a mature VEGF-C protein, it is clear that the residues of SEQ ID NO: 33 as originally filed were misnumbered by one residue. Because the error and its proper correction are apparent from the specification as originally filed, the corrections to SEQ ID NOs: 32-33 do not introduce new matter.

SEQ ID NOs: 44-45 of the substitute Sequence Listing depict a 1997 base pair nucleotide sequence and a deduced amino acid sequence of a cDNA that was deposited with the ATCC and cross-referenced at pp. 28-29 of the patent application as filed. These sequences are inherent properties of the deposited plasmid and thus find support in the deposited plasmid itself. See *Kennecott Corp. v. Kyocera International Inc.* 5 U.S.P.Q.2d 1194 (Fed. Cir. 1987) (The express description of an inherent property is not new matter and can be added to a specification with effect as of the original filing date); *In re Lundak*, 227 U.S.P.Q. 90 (Fed. Cir. 1985); see also Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo (filed herewith) at ¶¶ 2-5.

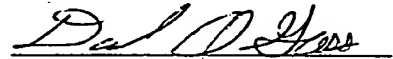
In accordance with 37 C.F.R. §1.68, I hereby declare that the foregoing statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, IL 60606-6402
Telephone: (312) 474-6300

November 26, 1997


David A. Gass
Registration No. 38,153



PATENT
28967/33072

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996


Title: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

) I hereby certify that this paper is being
) deposited with the United States Postal
) Service as first class mail, postage
) prepaid, in an envelope addressed to:
) Assistant Commissioner for Patents
) Washington, D.C. 20231, on this date:

) Dated: Nov. 26, 1997

) 
) David A. Gass
) Registration No. 38,153

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. KARI ALITALO

1. I am a co-inventor of the above-identified U.S. Patent Application (hereinafter "the patent application"). I am familiar with the Office action from the U.S. Patent and Trademark Office dated May 28, 1997, in the patent application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the issues and rejections raised in the Office action.

Isolation of VEGF-C protein and cDNA

2. The present invention relates generally to a protein ligand for Flt4 receptor tyrosine kinase (VEGFR-3), which our research team has designated "VEGF-C." As taught in Example 14 of the patent application, VEGF-C also stimulates KDR/Flk-1 receptor tyrosine kinase (VEGFR-2). Our research team purified a VEGF-C protein that we discovered in conditioned media from a PC-3 prostatic adenocarcinoma cell line. We demonstrated that this protein bound to the extracellular domain of Flt4 and stimulated Flt4 phosphorylation. (See the patent application at Examples 4-5, for example.) Using SDS polyacrylamide gel electrophoresis, the VEGF-C protein was originally determined to have a molecular weight of about 23 kilodaltons. This measurement is in good

agreement with subsequent measurements of VEGF-C that we have recombinantly expressed in multiple cell lines, where we have determined the molecular weight to be about 21-23 kD.)

3. We sequenced the amino terminus of this purified VEGF-C protein as taught in the patent application in Example 5. (See especially p. 23.) I hereby reaffirm that our sequencing data from this protein is correctly reported in the patent application at p. 23 and in SEQ ID NO: 13.

4. As taught in Examples 6-10 of the patent application, we used the amino terminal amino acid sequence taught in the patent application to obtain a cDNA encoding VEGF-C. A plasmid containing the cDNA that is described in Example 11 of the patent application was deposited with the American Type Culture Collection and accorded ATCC accession number 97231.

5. The patent application describes a partial nucleotide sequence and a 350 amino acid open reading frame of the deposited VEGF-C cDNA. (See SEQ ID NOs: 32 and 33 of the patent application.) In the amendment filed herewith, these sequences have been amended such that the designation of residue "1" therein corresponds with the first residue of VEGF-C purified from PC-3 conditioned medium as described in the patent application. (See also paragraph 3, above.) Amended SEQ ID NOs: 32-33 are attached hereto as Exhibit A. Complete sequencing of the cDNA subsequently demonstrated that the translated open reading frame is actually 419 amino acids: it extends 69 codons upstream of what is reported in SEQ ID NO: 33. Attached hereto as Exhibit B is a 1997 nucleotide sequence of the cDNA that was deposited with the ATCC. Exhibit B also depicts the deduced 419 amino acid open reading frame. These sequences have been added to the patent application as SEQ ID NOs: 44 and 45. I shall use the term "prepro-VEGF-C" herein to refer to a polypeptide consisting of this 419 amino acid sequence.

6. As taught in the patent application (e.g., at p. 11), the carboxyl-terminal amino acid sequences encoded by the VEGF-C cDNA show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring 3 protein (BR3P) sequence that was

known in the art. (See Dignam and Case, *Gene*, 88:133-40 (1990); and Paulsson, *et al.*, *J. Mol. Biol.*, 211:331-49 (1990), both of record and cited in the patent application). The distinctive BR3P cysteine motifs (Cys-Xaa_n-Cys-Xaa-Cys-Xaa-Cys, wherein Xaa is any residue and n is variable) occur at least four times in the carboxy-terminal portion of VEGF-C (see Cys residues in Exhibit B at positions 280, 291, 293, and 295; positions 304, 315, 317, and 319; positions 328, 339, 341, and 343; and positions 347, 358, 360, and 362).

**VEGF-C processing and determination of
VEGF-C fragments that bind to Flt4.**

7. The Patent application teaches that the protein encoded by the VEGF-C gene is proteolytically processed, and teaches procedures to characterize this processing, such as analysis using antibodies and pulse-chase experiments. The application further teaches to screen truncated forms of VEGF-C (e.g., deletion fragments) to determine the portions of VEGF-C that are necessary to bind and stimulate Flt4. (See, e.g., pp. 29-30 of the patent application.) Using techniques such as those described at pp. 29-30 of the patent application and mutational analysis, our research team has extensively characterized the processing of human prepro-VEGF-C in mammalian cell lines.

A. Our results from pulse-chase experiments indicate that the apparent first proteolytic processing of human prepro-VEGF-C involves cleavage of a signal peptide of about 31 residues, leaving residues 32-419 (hereinafter "pro-VEGF-C"). Pro-VEGF-C has an apparent molecular weight of about 55-58 kD.

B. We next observed that pro-VEGF-C is cleaved, either intracellularly or at the cell surface, into polypeptides of about 29 kD and about 31-32 kD (when assessed by SDS-PAGE under reducing conditions). The ~32 kD polypeptide binds the extracellular domain of Flt4 receptor tyrosine kinase with high affinity. (See Example 13 of the patent application.) The ~32 kD polypeptide was purified with immunoaffinity chromatography using an anti-VEGF-C antibody. The amino-terminus of

this purified polypeptide was determined to correspond to position 32 of the sequence shown in Exhibit B. Thus, the ~32 kD polypeptide represents the amino-terminal product of this proteolytic cleavage. Sequencing of the ~29 kD polypeptide indicated that cleavage occurred after amino acid 227 of the 419 amino acid sequence depicted in Exhibit B. (Amino acid 227 corresponds to residue 125 of SEQ ID NO: 33 in the patent application (Exhibit A).) This carboxy-terminal fragment of about 29 kD presumably includes residues 228-419 of the sequence depicted in Exhibit B (residues 126-317 of SEQ ID NO: 33). Thus, the ~29 kD polypeptide includes all of the Balbiani ring 3 protein cysteine motifs of VEGF-C (see paragraph 6 above). These results indicate that polypeptide fragments of the sequences depicted in Exhibits A or B that lack any domain having cysteine motifs of a Balbiani ring 3 protein (e.g., that lack the ~29 kD carboxy-terminal fragment) remain capable of binding with the extracellular domain of Flt4.

C. We also have observed forms of VEGF-C that reflect further proteolytic processing at the amino terminus. For the purpose of this declaration, I shall collectively refer to forms of VEGF described below as "mature VEGF-C."

- i. As indicated in paragraph 3, above, VEGF-C isolated from conditioned medium of PC-3 cells has an amino terminus corresponding to amino acid 103 in Exhibit B (i.e., amino acid 1 of SEQ ID NO: 33 (Exhibit A)).
- ii. We have sequenced VEGF-C that was recombinantly expressed in 293-EBNA cells (as described in Example 11 of the patent application) and determined that the amino terminus of this form corresponds with position 112 of the sequence shown in Exhibit B (i.e., position 10 of SEQ ID NO: 33 (Exhibit A)).

8. Our research team modified the human VEGF-C cDNA to recombinantly produce a fragment consisting of amino acids 104-213 of the 419 amino acid polypeptide in yeast (i.e., residues 2-111 of SEQ ID NO: 33). This fragment was shown to bind Flt4 and stimulate phosphorylation of both Flt4 (VEGFR-3) and KDR (VEGFR-2). In another experiment, a fragment lacking residues 1-112 of the 419 amino acid polypeptide retained receptor binding activity.

9. Collectively, the experimental results described in the preceding paragraphs indicate that polypeptides lacking amino acids 1-112 and 214-419 of the 419 residue amino acid sequence shown in Exhibit B retain Flt4 binding and stimulating activities. Stated differently, we have experimental evidence to indicate that a polypeptide corresponding to positions 11-112 of SEQ ID NO: 33 will retain Flt4 binding and stimulating activities. Moreover, one skilled in the art understands from the patent application how to perform receptor binding and phosphorylation assays, to localize further the portion of SEQ ID NO: 33 that is required for activity.

**The application enables one to obtain
VEGF-C-encoding cDNAs from non-human sources**

10. I infer from page 5 of the Office action that the Patent Office has rejected a claim of the application in part because of the lack of a claim limitation with respect to the source animal for VEGF-C. This section of the declaration provides evidence that the teachings in the patent application of a human VEGF-C cDNA, combined with the teachings that VEGF-C protein binds Flt4 (VEGFR-3) and VEGFR-2, enable one to obtain VEGF-C-encoding cDNAs from non-human sources.

11. To clone a murine VEGF-C cDNA, approximately 1×10^6 bacteriophage lambda clones of a commercially-available 12 day mouse embryonal cDNA library (lambda EXlox library, Novagen, catalog number 69632-1) were screened with a radiolabeled fragment of human VEGF-C cDNA containing nucleotides 495 to 1661 of the nucleotide sequence shown in Exhibit B. One positive clone was isolated.

12. A 1323 bp *EcoRI/HindIII* fragment of the insert of the isolated mouse cDNA clone was subcloned into the corresponding sites of the pBluescript SK+ vector (Stratagene) and sequenced. The cDNA sequence of this clone was homologous to the human VEGF-C sequence reported herein, except that about 710 bp of 5'-end sequence present in the human clone was not present in the mouse clone.

13. For further screening of mouse cDNA libraries, a *HindIII-BstXI* (*HindIII* site is from the pBluescript SK+ polylinker) fragment of 881 bp from the coding region of the mouse cDNA clone was radiolabeled and used as a probe to screen two additional mouse cDNA libraries. Two additional cDNA clones from an adult mouse heart ZAP II cDNA library (Stratagene, catalog number 936306) were identified. Three additional clones also were isolated from a mouse heart 5'-stretch-plus cDNA library in λ gt11 (Clontech Laboratories, Inc., catalog number ML5002b). Of the latter three clones, one was found to contain an insert of about 1.9 kb. The insert of this cDNA clone was subcloned into *EcoRI* sites of pBluescript SK+ vector and both strands of this clone were completely sequenced, resulting in the nucleotide and deduced amino acid sequences shown in Exhibit C. It is expected that the mouse VEGF-C polypeptide depicted in Exhibit C is processed into a mature mouse VEGF-C protein, in a manner analogous to the processing of the human prepro-VEGF-C.

14. The foregoing results demonstrate the utility of human VEGF-C-encoding polynucleotides of the invention for identifying and isolating polynucleotides encoding other non-human mammalian VEGF-C proteins. Such identified and isolated polynucleotides, in turn, can be expressed (using procedures similar to those described in the patent application for human VEGF-C) to produce recombinant polypeptides corresponding to non-human mammalian forms of VEGF-C.

15. The identity of the mouse protein as VEGF-C was confirmed by recombinantly expressing the above-described mouse cDNA, and analyzing the expressed proteins.

A. The 1.8 kb mouse VEGF-C cDNA was cloned as an *EcoRI* fragment into the retroviral expression vector pBabe-puro containing the SV40 early promoter region [Morgenstern *et al.*, *Nucl. Acids Res.*, 18:3587-3595 (1990)], and transfected into the Bosc23 packaging cell line [Pearet *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 90:8392-8396 (1994)] by the calcium-phosphate precipitation method. For comparison, Bosc23 cells also were transfected with the previously-described human VEGF-C construct in the pREP7 expression vector. The expressed proteins were immunoprecipitated with polyclonal antibodies raised against mature human VEGF-C.

B. Immunoprecipitation of VEGF-C from media of transfected and metabolically-labelled cells revealed bands of approximately $30\text{-}32 \times 10^3 M_r$ (a doublet) and $22\text{-}23 \times 10^3 M_r$ in 12.5% SDS-PAGE. These bands were not detected in samples from nontransfected or mock-transfected cells. These results demonstrate that antibodies raised against human VEGF-C recognize the corresponding mouse protein.

C. For receptor binding experiments, 1 ml aliquots of media from metabolically-labelled Bosc23 cells were incubated with VEGFR-3 extracellular domain, covalently coupled to sepharose, for 4 hours at 4°C with gentle mixing. (See Examples 4 and 5 in the patent application.) The sepharose beads were washed four times with ice-cold phosphate buffered saline (PBS), and the samples were analyzed by gel electrophoresis as described in Joukov *et al.*, *EMBO J.*, 15:290-298 (1996).

D. Similar $30\text{-}32 \times 10^3 M_r$ doublet and $22\text{-}23 \times 10^3 M_r$ polypeptide bands were obtained in the receptor binding assay as compared to the immunoprecipitation assay. In additional experiments, mouse VEGF-C appeared to be a potent inducer of VEGFR-3 autophosphorylation, too. Thus, the putative mouse VEGF-C binds and stimulates human VEGFR-3, confirming its identity. The slightly faster mobility of the mouse VEGF-C polypeptides that was observed may be caused by the four amino acid

residue difference observed in sequence analysis (residues H88-E91).

Murine VEGF-C appeared to bind VEGFR-2 with lower affinity.

16. The human VEGF-C cDNA also was used to design probes for successfully isolating a quail VEGF-C cDNA from a quail cDNA library. A fragment of the human VEGF-C cDNA comprising nucleotides 495-1661 of Exhibit B was obtained by PCR amplification, cloned into the pCRII vector (Invitrogen) according to the manufacturer's instructions, and amplified. The insert was isolated by *Eco* RI digestion and preparative gel electrophoresis and then labelled using radioactive dCTP and random priming. A cDNA library made from quail embryos of stage E-4 in pcDNA-1 vector (Invitrogen) was then screened using this probe. About 200,000 colonies were plated and filter replicas were hybridized with the radioactive probe under reduced stringency conditions (washes at 42°C with a wash solution comprising 2x SSC/0.1% SDS). Nine positive clones were identified and secondarily plated. Two of the nine clones hybridized in secondary screening. The purified clones (clones 1 and 14) had approximately 2.7 kb *Eco* RI inserts. Both clones were amplified and then sequenced using the T7 and SP6 primers (annealing to the vector). In addition, an internal *Sph* I restriction endonuclease cleavage site was identified about 1.9 kb from the T7 primer side of the vector and used for subcloning 5'- and 3'- *Sph* I fragments, followed by sequencing from the *Sph* I end of the subclones. The sequences obtained were identical from both clones and showed a high degree of similarity to the human VEGF-C coding region. Subsequently, walking primers were made in both directions and double-stranded sequencing was completed for 1743 base pairs, including the full-length open reading frame.

17. The cDNA sequence obtained includes a long open reading frame and 5' untranslated region. The DNA and deduced amino acid sequences for the quail cDNA are set forth in Exhibit D. Studies performed with the putative quail VEGF-C cDNA have shown that its protein product is secreted from transfected cells and interacts with avian VEGFR-3 and VEGFR-2, further confirming the conclusion that the cDNA encodes a quail VEGF-C protein.

18. As shown in Exhibit E, the human, murine, and avian (quail) VEGF-C precursor amino acid sequences share a significant degree of conservation. This high degree of homology confirms the likelihood of success of attempts to isolate VEGF-C encoding sequences from other species, especially vertebrate species, and more particularly mammalian and avian species, using human VEGF-C-encoding polynucleotides taught in the patent application as probes and using standard molecular biological techniques. The identity of putative VEGF-C-encoding cDNAs is confirmed using receptor binding studies such as the studies described in the patent application.

Certification

19. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

November 20, 1997
Date

Jan Alitalo
Kari Alitalo

EXHIBIT A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAAC	ATG ACT GTA CTC TAC CCA	54
	Met Thr Val Leu Tyr Pro	
	-33 -30	
GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA	102	
Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Trp Gln		
	-25 -20 -15	
CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA	150	
His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile		
	-10 -5 1 5	
AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT	198	
Lys Phe Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp		
	10 15 20	
AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT	246	
Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp		
	25 30 35	
GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA	294	
Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro		
	40 45 50	
TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG	342	
Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu		
	55 60 65	
CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA	390	
Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu		
	70 75 80 85	
ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT	438	
Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe		
	90 95 100	
GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA	486	
Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg		
	105 110 115	
CRA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG	534	
Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln		
	120 125 130	
TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT	582	
Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn		
	135 140 145	
CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT	630	
His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp		
	150 155 160 165	
GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC	678	
Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn		
	170 175 180	
AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CIT	726	
Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu		
	185 190 195	
CGG CCT GCC ACC TGT GGA GCG CAC AAA GAA CTA GAC AGA AAC TCA TGC	774	
Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys		
	200 205 210	

200										205										210										
CAG	TGT	GTC	TGT	AAA	AAC	AAA	CTC	TTC	CCC	AGC	CAA	TGT	GGG	GCC	AAC	822														
Gln	Cys	Val	Cys	Lys	Asn	Lys	Leu	Phe	Pro	Ser	Gln	Cys	Gly	Ala	Asn															
215						220					225																			
CGA	GAA	TTT	GAT	GAA	AAC	ACA	TGC	CAG	TGT	GTA	TGT	AAA	AGA	ACC	TGC	870														
Arg	Glu	Phe	Asp	Glu	Asn	Thr	Cys	Gln	Cys	Val	Cys	Lys	Arg	Thr	Cys															
230					235					240					245															
CCC	AGA	AAT	CAA	CCC	CTA	AAT	CCT	GGA	AAA	TGT	GCC	TGT	GAA	TGT	ACA	918														
Pro	Arg	Asn	Gln	Pro	Leu	Asn	Pro	Gly	Lys	Cys	Ala	Cys	Glu	Cys	Thr															
				250					255					260																
GAA	AGT	CCA	CAG	AAA	TGC	TTG	TTA	AAA	GGA	AAG	AAG	TTC	CAC	CAC	CAA	966														
Glu	Ser	Pro	Gln	Lys	Cys	Leu	Leu	Lys	Gly	Lys	Lys	Phe	His	His	Gln															
				265				270					275																	
ACA	TGC	AGC	TGT	TAC	AGA	CGG	CCA	TGT	ACG	AAC	CGC	CAG	AAG	GCT	TGT	1014														
Thr	Cys	Ser	Cys	Tyr	Arg	Arg	Pro	Cys	Thr	Asn	Arg	Gln	Lys	Ala	Cys															
				280			285					290																		
GAG	CCA	GGA	TTT	TCA	TAT	AGT	GAA	GAA	GTG	TGT	CGT	TGT	GTC	CCT	TCA	1062														
Glu	Pro	Gly	Phe	Ser	Tyr	Ser	Glu	Glu	Val	Cys	Arg	Cys	Val	Pro	Ser															
				295		300					305																			
TAT	TGG	AAA	AGA	CCA	CAA	ATG	AGC	TAAGATTGTA	CTGTTTTC	CA	GTTTCATCGAT					1116														
Tyr	Trp	Lys	Arg	Pro	Gln	Met	Ser																							
310					315																									
TTTCTATTAT GGAAAACGTGT GTTG																1140														

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 350 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met	Thr	Val	Leu	Tyr	Pro	Glu	Tyr	Trp	Lys	Met	Tyr	Lys	Cys	Gln	Leu
-33			-30					-25					-20		
Arg	Lys	Gly	Gly	Trp	Gln	His	Asn	Arg	Glu	Gln	Ala	Asn	Leu	Asn	Ser
	-15						-10					-5			
Arg	Thr	Glu	Glu	Thr	Ile	Lys	Phe	Ala	Ala	Ala	His	Tyr	Asn	Thr	Glu
	1				5				10						15
Ile	Leu	Lys	Ser	Ile	Asp	Asn	Glu	Trp	Arg	Lys	Thr	Gln	Cys	Met	Pro
			20					25						30	
Arg	Glu	Val	Cys	Ile	Asp	Val	Gly	Lys	Glu	Phe	Gly	Val	Ala	Thr	Asn
			35				40						45		
Thr	Phe	Phe	Lys	Pro	Pro	Cys	Val	Ser	Val	Tyr	Arg	Cys	Gly	Gly	Cys
			50				55					60			
Cys	Asn	Ser	Glu	Gly	Leu	Gln	Cys	Met	Asn	Thr	Ser	Thr	Ser	Tyr	Leu
			65			70					75				
Ser	Lys	Thr	Leu	Phe	Glu	Ile	Thr	Val	Pro	Leu	Ser	Gln	Gly	Pro	Lys
			80			85				90					95

Pro	Val	Thr	Ile	Ser	Phe	Ala	Asn	His	Thr	Ser	Cys	Arg	Cys	Met	Ser	100	105	110
Lys	Leu	Asp	Val	Tyr	Arg	Gln	Val	His	Ser	Ile	Ile	Arg	Arg	Ser	Leu	115	120	125
Pro	Ala	Thr	Leu	Pro	Gln	Cys	Gln	Ala	Ala	Asn	Lys	Thr	Cys	Pro	Thr	130	135	140
Asn	Tyr	Met	Trp	Asn	Asn	His	Ile	Cys	Arg	Cys	Leu	Ala	Gln	Glu	Asp	145	150	155
Phe	Met	Phe	Ser	Ser	Asp	Ala	Gly	Asp	Asp	Ser	Thr	Asp	Gly	Phe	His	160	165	170
Asp	Ile	Cys	Gly	Pro	Asn	Lys	Glu	Leu	Asp	Glu	Glu	Thr	Cys	Gln	Cys	180	185	190
Val	Cys	Arg	Ala	Gly	Leu	Arg	Pro	Ala	Ser	Cys	Gly	Pro	His	Lys	Glu	195	200	205
Leu	Asp	Arg	Asn	Ser	Cys	Gln	Cys	Val	Cys	Lys	Asn	Lys	Leu	Phe	Pro	210	215	220
Ser	Gln	Cys	Gly	Ala	Asn	Arg	Glu	Phe	Asp	Glu	Asn	Thr	Cys	Gln	Cys	225	230	235
Val	Cys	Lys	Arg	Thr	Cys	Pro	Arg	Asn	Gln	Pro	Leu	Asn	Pro	Gly	Lys	240	245	250
Cys	Ala	Cys	Glu	Cys	Thr	Glu	Ser	Pro	Gln	Lys	Cys	Leu	Leu	Lys	Gly	260	265	270
Lys	Lys	Phe	His	His	Gln	Thr	Cys	Ser	Cys	Tyr	Arg	Arg	Pro	Cys	Thr	275	280	285
Asn	Arg	Gln	Lys	Ala	Cys	Glu	Pro	Gly	Phe	Ser	Tyr	Ser	Glu	Glu	Val	290	295	300
Cys	Arg	Cys	Val	Pro	Ser	Tyr	Trp	Lys	Arg	Pro	Gln	Met	Ser			305	310	315

EXHIBIT B

CCCCCCCCGC	CTCTCCAAAA	AGCTACACCG	ACGCGGACCG	CGGCGGCGTC	CTCCCTCGCC	60
CTCGCTTCAC	CTCGCGGGCT	CCGAATGCGG	GGAGCTCGGA	TGTCCGGTTT	CCTGTGAGGC	120
TTTACTCTGA	CACCCGCCGC	CTTTCCCGG	CACTGGCTGG	GAGGGCGCCC	TGCAAAGTTG	180
GGAACGCGGA	GCCCCGGACC	CGCTCCCGCC	GCCTCCGGCT	CGCCAGGGG	GGTCTGCCGG	240
GAGGAGCCCG	GGGGAGAGGG	ACCAGGAGGG	GCCCCGCGCC	TQCGAGGGG	GCCCCGCGCC	300
CCACCCCTGC	CCCCGCCAGC	GGACCGGTCC	CCCACCCCG	GTCTTCCAC	C ATG CAC Met His 1	357
TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG	Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu	5 10 15	405			
CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC TTC GAG TCC	Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser	20 25 30	453			
GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT	Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala	35 40 45 50	501			
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA	Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val	55 60 65	549			
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG	Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys	70 75 80	597			
TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC	Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Ala Ala Gln Ala Asn	85 90 95	645			
CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT	Leu Asn Ser Arg Thr Glu Thr Ile Lys Phe Ala Ala Ala His Tyr	100 105 110	693			
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA	Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln	115 120 125 130	741			
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC	Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val	135 140 145	789			
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT	Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys	150 155 160	837			
GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG	Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr	165 170 175	885			
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA	Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln	180 185 190	933			
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA	Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg	195 200 205 210	981			

TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg 215 220 225	1029
CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr 230 235 240	1077
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala 245 250 255	1125
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp 260 265 270	1173
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr 275 280 285 290	1221
TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro 295 300 305	1269
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys 310 315 320	1317
CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr 325 330 335	1365
TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn 340 345 350	1413
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu 355 360 365 370	1461
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg 375 380 385	1509
CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser 390 395 400	1557
GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met 405 410 415	1605
AGC TAAGATTGTA CTGTTTCCCA GTTCATCGAT TTTCTATTAT GGAAACTGT Ser	1658
GTGGCCACAG TAGAACTGTC TGTGAACAGA GAGACCCTTG TGGGTCCATG CTAACAAAGA	1718
CAAAAGTCTG TCTTTCCTGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGCTCATC	1778
TGCAAAAGGC CTCTTGTAAG GACTGGTTTT CTGCCAATGA CCAACACGCC AAGATTTTCC	1838
TCTTGATGATT TCTTTAAAG AATGACTATA TAATTIATTT CCACTAAAAA TATTGTTTCT	1898
GCAITCATTT TTATAGCAAC AACAATTGGT AAAACTCACT GTGATCAATA TTTTATATC	1958
ATGCAAAATA TGTTTAAAT AAAATGAAAA TTGTATTAT	1997

EXHIBIT C

Mouse VEGF-C cDNA and deduced amino acid sequence

GCGGCCGCGT	CGACGCAAAA	GTTCGAGGCC	GCCGAGTCCC	GGGAGACGCT	CGCCCAGGGG	60
GGTCCCCGGG	AGGAAACCAC	GGGACAGGGA	CCAGGAGAGG	ACCTCAGCCT	CACGCCCCAG	120
CCTGCGCCAG	CCAACGGACC	GGCCTCCCTG	CTCCCGGTCC	ATCCACC	ATG CAC TTG Met His Leu	176
					1	
CTG TGC TTC TTG TCT CTG GCG TGT TCC CTG CTC GCC GCT GCG CTG ATC						224
Leu Cys Phe Leu Ser Leu Ala Cys Ser Leu Leu Ala Ala Ala Leu Ile						
	5		10		15	
CCC AGT CCG CGC GAG GCG CCC GCC ACC GTC GCC GCC TTC GAG TCG GGA						272
Pro Ser Pro Arg Glu Ala Pro Ala Thr Val Ala Ala Phe Glu Ser Gly						
	20		25		30	35
CTG GGC TTC TCG GAA GCG GAG CCC GAC GGG GGC GAG GTC AAG GCT TTT						320
Leu Gly Phe Ser Glu Ala Glu Pro Asp Gly Glu Val Lys Ala Phe						
		40		45	50	
GAA GGC AAA GAC CTG GAG GAG CAG TTG CGG TCT GTG TCC AGC GTA GAT						368
Glu Gly Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Val Asp						
		55		60	65	
GAG CTG ATG TCT GTC CTG TAC CCA GAC TAC TGG AAA ATG TAC AAG TGC						416
Glu Leu Met Ser Val Leu Tyr Pro Asp Tyr Trp Lys Met Tyr Lys Cys						
	70		75		80	
CAG CTG CGG AAA GGC GGC TGG CAG CAG CCC ACC CTC AAT ACC AGG ACA						464
Gln Leu Arg Lys Gly Gly Trp Gln Gln Pro Thr Leu Asn Thr Arg Thr						
	85		90		95	
GGG GAC AGT GTA AAA TTT GCT GCT GCA CAT TAT AAC ACA GAG ATC CTG						512
Gly Asp Ser Val Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu						
	100		105		110	115
AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGT GAG						560
Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu						
		120		125	130	
GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GCA GCC ACA AAC ACC TTC						608
Val Cys Ile Asp Val Gly Lys Glu Phe Gly Ala Ala Thr Asn Thr Phe						
		135		140	145	
TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAC						656
Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn						
	150		155		160	
AGC GAG GGG CTG CAG TGC ATG AAC ACC AGC ACA GGT TAC CTC AGC AAG						704
Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Gly Tyr Leu Ser Lys						
	165		170		175	
ACG TTG TTT GAA ATT ACA GTG CCT CTC TCA CAA GGC CCC AAA CCA GTC						752
Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val						
	180		185		190	195
ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGG TGC ATG TCT AAA CTG						800
Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu						
		200		205	210	
GAT GTT TAC AGA CAA GTT CAT TCA ATT ATT AGA CGT TCT CTG CCA GCA						848
Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala						
		215		220	225	

ACA TTA CCA CAG TGT CAG GCA GCT AAC AAG ACA TGT CCA ACA AAC TAT Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr 230 235 240	896
GTG TGG AAT AAC TAC ATG TGC CGA TGC CTG GCT CAG CAG GAT TTT ATC Val Trp Asn Asn Tyr Met Cys Arg Cys Leu Ala Gln Gln Asp Phe Ile 245 250 255	944
TTT TAT TCA AAT GTT GAA GAT GAC TCA ACC AAT GGA TTC CAT GAT GTC Phe Tyr Ser Asn Val Glu Asp Asp Ser Thr Asn Gly Phe His Asp Val 260 265 270 275	992
TGT GGA CCC AAC AAG GAG CTG GAT GAA GAC ACC TGT CAG TGT GTC TGC Cys Gly Pro Asn Lys Glu Leu Asp Glu Asp Thr Cys Gln Cys Val Cys 280 285 290	1040
AAG GGG GGG CTT CGG CCA TCT AGT TGT GGA CCC CAC AAA GAA CTA GAT Lys Gly Gly Leu Arg Pro Ser Ser Cys Gly Pro His Lys Glu Leu Asp 295 300 305	1088
AGA GAC TCA TGT CAG TGT GTC TGT AAA AAC AAA CTT TTC CCT AAT TCA Arg Asp Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Asn Ser 310 315 320	1136
TGT GGA GCC AAC AGG GAA TTT GAT GAG AAT ACA TGT CAG TGT GTA TGT Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys 325 330 335	1184
AAA AGA ACG TGT CCA AGA AAT CAG CCC CTG AAT CCT GGG AAA TGT GCC Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala 340 345 350 355	1232
TGT GAA TGT ACA GAA AAC ACA CAG AAG TGC TTC CTT AAA GGG AAG AAG Cys Glu Cys Thr Gln Asn Thr Gln Lys Cys Phe Leu Lys Gly Lys Lys 360 365 370	1280
TTC CAC CAT CAA ACA TGC AGT TGT TAC AGA AGA CCG TGT GCG AAT CGA Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Ala Asn Arg 375 380 385	1328
CTG AAG CAT TGT GAT CCA GGA CTG TCC TTT AGT GAA GAA GTA TGC CGC Leu Lys His Cys Asp Pro Gly Leu Ser Phe Ser Glu Glu Val Cys Arg 390 395 400	1376
TGT GTC CCA TCG TAT TGG AAA AGG CCA CAT CTG AAC TAAGATCATA Cys Val Pro Ser Tyr Trp Lys Arg Pro His Leu Asn 405 410 415	1422
CCAGTTTTC GTCAGTCACA GTCATTTACT CTCCTGAAGA CTGTTGGAAC AGCACTTAGC	1482
ACTGTCTATG CACAGAAAGA CTCGTGGGA CCACATGGTA ACAGAGGCCC AAGTCTGTGT	1542
TTATTGAACC ATGTGGATTA CTGCGGGAGA GGAAGTGGCAC TCATGTGCAA AAAAAACCTC	1602
TTCAAAGACT GGTTTTCTGC CAGGGACCAG ACAGCTGAGG TTTTCTCTTT GTGATTTAAA	1662
AAAAGAATGA CTATATAATT TATTTCCACT AAAAATATTG TTCCTGCATT CATTTTTATA	1722
GCAATAACAA TTGGTAAAGC TCACTGTGAT CAGTATTTTT ATAACATGCA AACTATGTT	1782
TAAATAAAAA TGAAAATTGT ATTATAAAAA AAAAAAAAAA AAAAAAAAAA GCTT	1836

EXHIBIT D

Quail VEGF-C

GCCCCCGCCG AGCGCTCCGC GCGCAGCGCG CGGGCCGGGC CGGCCCGCGG AGGGCGCGCT	60
GCGAGCGGCC ACTGGGTCCT GCTTCCCTCC TTCTCTCTCC TCCTCTCTCT CCTCCTTCTC	120
TCTGCGCTTT CCACCGCTCC CGAGCGAGCG CACGCTCGGA TGTCGGTTTT CCTGGTGGGT	180
TTTTTACCTG GCAAAGTCCG GATAACTTCG GTGAGAAATT GCAAAGAGGC TGGGAGCTCC	240
CCTGCAGGCG TCTGGGAGCT GCTGCCGCCG TCGCATCTTC TCCTATCCCG GGATTTTACT	300
GCCTTGATA TTGCGAGGGG AGGGAGGGGG GTGAGGACAG CAAAAAGAAA GGGGTGGGGG	360
GGGGGAGAGA AAAGGAAAAG AAGGAGCCTC GGAATTGTGC CCGCATTCCT GCGTGCCCCC	420
GCGGCCCCCC TCCGCTCTGC CATCTCCGCA CA ATG CAC TTG CTG GAG ATG CTC	473
Met His Leu Leu Glu Met Leu	
1 5	
TCC CTG GGC TGC TGC CTC GCT GCT GGC GCC GTG CTC CTG GGA CCC CGG	521
Ser Leu Gly Cys Cys Leu Ala Ala Gly Ala Val Leu Leu Gly Pro Arg	
10 15 20	
CAG CCG CCC GTC GCC GCC GCC TAC GAG TCC GGG CAC GGC TAC TAC GAG	569
Gln Pro Val Ala Ala Tyr Glu Ser Gly His Gly Tyr Tyr Glu	
25 30 35	
GAG GAG CCC GGT GCC GGG GAA CCC AAG GCT CAT GCA AGC AAA GAC CTG	617
Glu Glu Pro Gly Ala Gly Glu Pro Lys Ala His Ala Ser Lys Asp Leu	
40 45 50 55	
GAA GAG CAG TTG CGA TCT GTG TCC AGT GTG GAT GAA CTC ATG ACA GTA	665
Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp Glu Leu Met Thr Val	
60 65 70	
CTT TAC CCA GAA TAC TGG AAA ATG TTC AAA TGT CAG TTG AGG AAA GGA	713
Leu Tyr Pro Glu Tyr Trp Lys Met Phe Lys Cys Gln Leu Arg Lys Gly	
75 80 85	
GGT TGG CAA CAC AAC AGG GAA CAC TCC AGC TCT GAT ACA AGA TCA GAT	761
Gly Trp Gln His Asn Arg Glu His Ser Ser Ser Asp Thr Arg Ser Asp	
90 95 100	
GAT TCA TTG AAA TTT GCC GCA GCA CAT TAT AAT GCA GAG ATC CTG AAA	809
Asp Ser Leu Lys Phe Ala Ala Ala His Tyr Asn Ala Glu Ile Leu Lys	
105 110 115	
AGT ATT GAT ACT GAA TGG AGA AAA ACC CAG GGC ATG CCA CGT GAA GTG	857
Ser Ile Asp Thr Glu Trp Arg Lys Thr Gln Gly Met Pro Arg Glu Val	
120 125 130 135	
TGT GTG GAT TTG GGG AAA GAG TTT GGA GCA ACT ACA AAC ACC TTC TTT	905
Cys Val Asp Leu Gly Lys Glu Phe Gly Ala Thr Thr Asn Thr Phe	
140 145 150	
AAA CCC CCG TGT GTA TCC ATC TAC AGA TGT GGA GGT TGC TGC AAT AGT	953
Lys Pro Pro Cys Val Ser Ile Tyr Arg Cys Gly Gly Cys Asn Ser	
155 160 165	
GAA GGA CTC CAG TGT ATG AAT ATC AGC ACA AAT TAC ATC AGC AAG ACA	1001
Glu Gly Leu Gln Cys Met Asn Ile Ser Thr Asn Tyr Ile Ser Lys Thr	
170 175 180	

TTG TTT GAG ATT ACA GTG CCT CTC TCT CAT GGC CCC AAA CCT GTA ACA	1049
Leu Phe Glu Ile Thr Val Pro Leu Ser His Gly Pro Lys Pro Val Thr	
185 190 195	
GTC AGT TTT GCC AAT CAC ACG TCC TGC CGA TGC ATG TCT AAG TTG GAT	1097
Val Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp	
200 205 210 215	
GTT TAC AGA CAA GTT CAT TCT ATC ATA AGA CGT TCC TTG CCA GCA ACA	1145
Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr	
220 225 230	
CAA ACT CAG TGT CAT GTG GCA AAC AAG ACC TGT CCA AAA AAT CAT GTC	1193
Gln Thr Gln Cys His Val Ala Asn Lys Thr Cys Pro Lys Asn His Val	
235 240 245	
TGG AAT AAT CAG ATT TGC AGA TGC TTA GCA CAG CAC GAT TTT GGT TTC	1241
Trp Asn Asn Gln Ile Cys Arg Cys Leu Ala Gln His Asp Phe Gly Phe	
250 255 260 265	
TCT TCT CAC CTT GGA GAT TCT GAC ACA TCT GAA GGA TTC CAT ATT TGT	1289
Ser Ser His Leu Gly Asp Ser Asp Thr Ser Glu Gly Phe His Ile Cys	
265 270 275	
GGG CCC AAC AAA GAG CTG GAT GAA GAA ACC TGT CAA TGC GTC TGC AAA	1337
Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Lys	
280 285 290 295	
GGA GGT GTG CGG CCC ATA AGC TGT GGC CCT CAC AAA GAA CTA GAC AGG	1385
Gly Gly Val Arg Pro Ile Ser Cys Gly Pro His Lys Glu Leu Asp Arg	
300 305 310	
GCA TCA TGT CAG TGC ATG TGC AAA AAC AAA CTG CTC CCC AGT TCC TGT	1433
Ala Ser Cys Gln Cys Met Cys Lys Asn Lys Leu Leu Pro Ser Ser Cys	
315 320 325	
GGG CCT AAC AAA GAA TTT GAT GAA GAA AAG TGC CAG TGT GTA TGT AAA	1481
Gly Pro Asn Lys Glu Phe Asp Glu Glu Lys Cys Gln Cys Val Cys Lys	
330 335 340	
AAG ACC TGT CCC AAA CAT CAT CCA CTA AAT CCT GCA AAA TGC ATC TGC	1529
Lys Thr Cys Pro Lys His His Pro Leu Asn Pro Ala Lys Cys Ile Cys	
345 350 355	
GAA TGT ACA GAA TCT CCC AAT AAA TGT TTC TTA AAA GGA AAA AGA TTT	1577
Glu Cys Thr Glu Ser Pro Asn Lys Cys Phe Leu Lys Gly Lys Arg Phe	
360 365 370 375	
CAT CAC CAG ACA TGC AGT TGT TAC AGA CCA CCA TGT ACA GTC CGA ACG	1625
His His Gln Thr Cys Ser Cys Tyr Arg Pro Pro Cys Thr Val Arg Thr	
380 385 390	
AAA CGC TGT GAT GCT GGA TTT CTG TTA GCT GAA GAA GTG TGC CGC TGT	1673
Lys Arg Cys Asp Ala Gly Phe Leu Leu Ala Glu Glu Val Cys Arg Cys	
395 400 405	
GTA CGC ACA TCT TGG AAA AGA CCA CTT ATG AAT TAAGCGAAGA AAGCACTACT	1726
Val Arg Thr Ser Trp Lys Arg Pro Leu Met Asn	
410 415	
CGCTATATAG TGTCG	1741

EXHIBIT E

VEGF-C alignment

	1				50
Hum	HMLLGFFSVA	CSLLAAALLP	GPREAPAAAA	AFESGLDLSL	AEPDAGEATA
Mou	MHLLCFLSLA	CSLLAAALIP	SPREAPATVA	AFESGLGFSE	AEPDGGEVKA
Qua	MHLEMLSLG	CCLAAGAVLL	GPRQPPVA.A	AYESGHGYE	EEPGEAGEPKA
	51				100
Hum	YASKDLEEQL	RSVSSVDELM	TVLYPEYWKH	YKQQLRKGGW	QHNREQANLN
Mou	FEGKDLEEQL	RSVSSVDELM	SVLYPDYWKH	YKQQLRKGGW	Q....QPTLN
Qua	HASKDLEEQL	RSVSSVDELM	TVLYPEYWKH	FKQQLRKGGW	QHNREHSSSD
	101				150
Hum	SRTEETIKFA	AAHYNTEILK	SIDNEWRTQ	CMPEVVCIDV	GKEFGVATNT
Mou	TRTGDSVKFA	AAHYNTEILK	SIDNEWRTQ	CMPEVVCIDV	GKEFGAATNT
Qua	TRSDDSLKFA	AAHYNAEILK	SIDTEWRTQ	GMPEVVCIDL	GKEFGATTNT
	151				200
Hum	FFKPPCVSVY	RCGGCCNSEG	LQCMNTSTSY	LSKTLFEITV	PLSQGPKPVT
Mou	FFKPPCVSVY	RCGGCCNSEG	LQCMNTSTGY	LSKTLFEITV	PLSQGPKPVT
Qua	FFKPPCVSIY	RCGGCCNSEG	LQCMNISTNY	ISKTLFEITV	PLSHGPKPVT
	201				250
Hum	ISFANHTSCR	CMSKLDVYRQ	VHSIIRSLP	ATLPQCCAAN	KTCPTNYMVN
Mou	ISFANHTSCR	CMSKLDVYRQ	VHSIIRSLP	ATLPQCCAAN	KTCPTNYMVN
Qua	VSFANHTSCR	CMSKLDVYRQ	VHSIIRSLP	ATQTQCHVAN	KTCPKNHVWN
	251				300
Hum	NHICRCLAQE	DFMFSSDAGD	DSTDGFHDIC	GPKNELDEET	CQCVCRAGLR
Mou	NYMCRCCLAQ	DFIFYSNVED	DSTNGFHDVC	GPKNELDEET	CQCVCCKGGLR
Qua	NQICRCLAQH	DFGFSHGLD	SDTSEGFHIC	GPKNELDEET	CQCVCCKGGVR
	301				350
Hum	PASCGPHKEL	DRNSCQCVC	NKLFPSCGA	NREFDENTCQ	CVCKRTCPRN
Mou	PSSCGPHKEL	DRDSCQCVC	NKLFPNSCGA	NREFDENTCQ	CVCKRTCPRN
Qua	PISCGPHKEL	DRASCQCMCK	NKLLPSSCGP	NKEFDEEKQ	CVCKKTCPKH
	351				400
Hum	QPLNPGKAC	ECTESPQKCL	LKGKKFHHQT	CSCYRRPCTN	RQKACEPGFS
Mou	QPLNPGKAC	ECTENTQKCF	LKGKKFHHQT	CSCYRRPCAN	RLKHCDPGLS
Qua	HPLNPAKCIC	ECTESPKNCF	LKGKRFHHQT	CSCYRRPCTV	RTKRCDAAGFL
	401		420		
Hum	YSEEVCRCP	SYWKRPMQMS*			
Mou	FSEEVCRCP	SYWKRPHLN.			
Qua	LAEEVCRCP	TSWKRPLMN*			

PAGE: 1

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895A

1812

DATE: 01/22/98
TIME: 15:36:47

INPUT SET: S22772.raw

This Raw Listing contains the General
Information Section and up to the first 5 pages.

SEQUENCE LISTING

ENTERED

1
2
3 (1) General Information:
4
5 (i) APPLICANT: Alitalo, Kari
6 Joukov, Vladimir
7
8 (ii) TITLE OF INVENTION: RECEPTOR LIGAND
9
10 (iii) NUMBER OF SEQUENCES: 45
11
12 (iv) CORRESPONDENCE ADDRESS:
13 (A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
14 (B) STREET: 6300 Sears Tower, 233 South Wacker Drive
15 (C) CITY: Chicago
16 (D) STATE: Illinois
17 (E) COUNTRY: United States of America
18 (F) ZIP: 60606-6402
19
20 (v) COMPUTER READABLE FORM:
21 (A) MEDIUM TYPE: Floppy disk
22 (B) COMPUTER: IBM PC compatible
23 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
24 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
25
26 (vi) CURRENT APPLICATION DATA:
27 (A) APPLICATION NUMBER: 08/585,895
28 (B) FILING DATE: 12-JAN-1996
29 (C) CLASSIFICATION:
30
31 (vii) PRIOR APPLICATION DATA:
32 (A) APPLICATION NUMBER: US 08/510,133
33 (B) FILING DATE: 01-AUG-1995
34
35 (viii) PRIOR APPLICATION DATA:
36 (A) APPLICATION NUMBER: US 08/340,011
37 (B) FILING DATE: 14-NOV-1994
38
39 (ix) ATTORNEY/AGENT INFORMATION:
40 (A) NAME: Gass, David A.
41 (B) REGISTRATION NUMBER: 38,153
42 (C) REFERENCE/DOCKET NUMBER: 28967/33072
43
44 (ix) TELECOMMUNICATION INFORMATION:
45 (A) TELEPHONE: 312/474-6300
46 (B) TELEFAX: 312/474-0448

PAGE: 2

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895A

DATE: 01/22/98
TIME: 15:36:49

INPUT SET: S22772.raw

```

47         (C) TELEX: 25-3856
48
49     (2) INFORMATION FOR SEQ ID NO:1:
50
51         (1) SEQUENCE CHARACTERISTICS:
52             (A) LENGTH: 20 base pairs
53             (B) TYPE: nucleic acid
54             (C) STRANDEDNESS: single
55             (D) TOPOLOGY: linear
56
57         (ii) MOLECULE TYPE: DNA (genomic)
58
59         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
60
61     TGTCTCTGCT GTCCTGTCT                                20
62
63     (2) INFORMATION FOR SEQ ID NO:2:
64
65         (1) SEQUENCE CHARACTERISTICS:
66             (A) LENGTH: 70 base pairs
67             (B) TYPE: nucleic acid
68             (C) STRANDEDNESS: single
69             (D) TOPOLOGY: linear
70
71         (ii) MOLECULE TYPE: DNA (genomic)
72
73         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
74
75     ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG    60
76
77     GACTCCTGGA                                            70
78
79     (2) INFORMATION FOR SEQ ID NO:3:
80
81         (1) SEQUENCE CHARACTERISTICS:
82             (A) LENGTH: 24 base pairs
83             (B) TYPE: nucleic acid
84             (C) STRANDEDNESS: single
85             (D) TOPOLOGY: linear
86
87         (ii) MOLECULE TYPE: DNA (genomic)
88
89         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
90
91     ACATGCATGC CCCGCCGTC ATCC                                24
92
93     (2) INFORMATION FOR SEQ ID NO:4:
94
95         (1) SEQUENCE CHARACTERISTICS:
96             (A) LENGTH: 22 base pairs
97             (B) TYPE: nucleic acid
98             (C) STRANDEDNESS: single
99             (D) TOPOLOGY: linear
```

INPUT SET: S22772.raw

100
101 (ii) MOLECULE TYPE: DNA (genomic)
102
103 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
104
105 CGGAATTCCC CATGACCCCA AC 22
106
107 (2) INFORMATION FOR SEQ ID NO:5:
108
109 (i) SEQUENCE CHARACTERISTICS:
110 (A) LENGTH: 33 base pairs
111 (B) TYPE: nucleic acid
112 (C) STRANDEDNESS: single
113 (D) TOPOLOGY: linear
114
115 (ii) MOLECULE TYPE: DNA (genomic)
116
117 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
118
119 CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT 33
120
121 (2) INFORMATION FOR SEQ ID NO:6:
122
123 (i) SEQUENCE CHARACTERISTICS:
124 (A) LENGTH: 17 base pairs
125 (B) TYPE: nucleic acid
126 (C) STRANDEDNESS: single
127 (D) TOPOLOGY: linear
128
129 (ii) MOLECULE TYPE: DNA (genomic)
130
131 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
132
133 ATTTAGGTGA CACTATA 17
134
135 (2) INFORMATION FOR SEQ ID NO:7:
136
137 (i) SEQUENCE CHARACTERISTICS:
138 (A) LENGTH: 34 base pairs
139 (B) TYPE: nucleic acid
140 (C) STRANDEDNESS: single
141 (D) TOPOLOGY: linear
142
143 (ii) MOLECULE TYPE: DNA (genomic)
144
145 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
146
147 CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT 34
148
149 (2) INFORMATION FOR SEQ ID NO:8:
150
151 (i) SEQUENCE CHARACTERISTICS:
152 (A) LENGTH: 40 amino acids

PAGE: 4

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895A

DATE: 01/22/99
TIME: 15:36:54

INPUT SET: S22772.raw

```
153 (B) TYPE: amino acid
154 (C) STRANDEDNESS: single
155 (D) TOPOLOGY: linear
156
157 (ii) MOLECULE TYPE: protein
158
159 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
160
161 Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
162 1 5 10 15
163
164 Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
165 20 25 30
166
167 His Arg Gln Glu Ser Gly Phe Arg
168 35 40
169
170 (2) INFORMATION FOR SEQ ID NO:9:
171
172 (i) SEQUENCE CHARACTERISTICS:
173 (A) LENGTH: 21 base pairs
174 (B) TYPE: nucleic acid
175 (C) STRANDEDNESS: single
176 (D) TOPOLOGY: linear
177
178 (ii) MOLECULE TYPE: DNA (genomic)
179
180 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
181
182 CTGGAGTCGA CTTGGCGGAC T
183
184 (2) INFORMATION FOR SEQ ID NO:10:
185
186 (i) SEQUENCE CHARACTERISTICS:
187 (A) LENGTH: 60 base pairs
188 (B) TYPE: nucleic acid
189 (C) STRANDEDNESS: single
190 (D) TOPOLOGY: linear
191
192 (ii) MOLECULE TYPE: DNA (genomic)
193
194 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
195
196 CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC
197
198 (2) INFORMATION FOR SEQ ID NO:11:
199
200 (i) SEQUENCE CHARACTERISTICS:
201 (A) LENGTH: 34 base pairs
202 (B) TYPE: nucleic acid
203 (C) STRANDEDNESS: single
204 (D) TOPOLOGY: linear
205
```

21

60

PAGE: 5

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895A

DATE: 01/22/98
TIME: 15:36:56

INPUT SET: S22772.raw

```
206 (ii) MOLECULE TYPE: DNA (genomic)
207
208 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
209
210 CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC 34
211
212 (2) INFORMATION FOR SEQ ID NO:12:
213
214 (i) SEQUENCE CHARACTERISTICS:
215 (A) LENGTH: 20 base pairs
216 (B) TYPE: nucleic acid
217 (C) STRANDEDNESS: single
218 (D) TOPOLOGY: linear
219
220 (ii) MOLECULE TYPE: DNA (genomic)
221
222 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
223
224 GTTGCCTGTG ATGTGCACCA 20
225
226 (2) INFORMATION FOR SEQ ID NO:13:
227
228 (i) SEQUENCE CHARACTERISTICS:
229 (A) LENGTH: 18 amino acids
230 (B) TYPE: amino acid
231 (C) STRANDEDNESS: single
232 (D) TOPOLOGY: linear
233
234 (ii) MOLECULE TYPE: peptide
235
236 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
237
238 Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
239 1 5 10 15
240
241 Leu Lys
242
243
244 (2) INFORMATION FOR SEQ ID NO:14:
245
246 (i) SEQUENCE CHARACTERISTICS:
247 (A) LENGTH: 17 base pairs
248 (B) TYPE: nucleic acid
249 (C) STRANDEDNESS: single
250 (D) TOPOLOGY: linear
251
252 (ii) MOLECULE TYPE: DNA (genomic)
253
254 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
255
256 GCAGARGARA CNATHAA 17
257
258 (2) INFORMATION FOR SEQ ID NO:15:
```

PAGE: 1

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION US/08/585,895A

DATE: 01/22/98
TIME: 15:36:59

INPUT SET: S22772.raw

Line

Error

Original Text



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	Title: RECEPTOR LIGAND
Alitalo et al.)	
Serial No. 08/585,895)	Art Unit: 1801
Filed: January 12, 1996)	Examiner: Lathrop, B.

Change of Inventor's Address

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:


Please be advised that the residence and mailing address of co-inventor Vladimir Joukov is now as follows:

51 Massachusetts Avenue, Apt. 1F
Boston, Massachusetts 02115

This notification is NOT intended as a change of correspondence address. Please continue to send correspondence to the Applicants' attorney at the address below:

Respectfully submitted,
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:


David A. Gass
Registration No. 38,153

Date: Feb 24, 1998

ASSIGNMENT

WHEREAS Helsinki University Licensing, Ltd., Viikinkaari 8 A, FIN-00710 Helsinki, Finland (hereinafter HUL), its successors and assigns, is the assignee of the entire right, title and interest in the invention or improvements of Kari Alitalo and Vladimir Joukov relating to the cloning, isolation and sequencing of human Vascular Endothelial Growth Factor C (VEGF-C) disclosed in certain applications for Letters Patent of the United States, and in said applications and any and all other applications, both United States and foreign, which Kari Alitalo and Vladimir Joukov may file, either solely or jointly with others, on said invention or improvements, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said applications, and in any reissue or extension thereof; and

WHEREAS, for ten dollars (\$10.00), and other good and valuable consideration enumerated in a written agreement dated 24 October 1996, the sufficiency of which is hereby acknowledged, HUL has agreed to share ownership of the aforementioned invention, improvements, applications, patents, reissues, extensions, and the like on a 50% / 50% equal basis with Ludwig Institute for Cancer Research, a Swiss not-for-profit corporation having an office at 1345 Avenue of the Americas, New York, New York 10105, United States of America (hereinafter LICR);

NOW, THEREFORE, HUL hereby assigns to LICR a fifty percent (50%) interest in the patent applications identified in the following LIST OF PATENT PROPERTIES, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said patent applications, and in any reissue or extension thereof.

LIST OF PATENT PROPERTIES

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>
08/510,133	01/08/95	Receptor Ligand
08/585,895	12/01/96	Receptor Ligand
08/601,132	14/02/96	Receptor Ligand
08/671,573	28/06/96	Receptor Ligand VEGF-C
PCT/FI96/00427	01/08/96	Receptor Ligand VEGF-C
08/795,430	02/05/97	Vascular Endothelial Growth Factor C (VEGF-C) Protein and Gene, Mutants Thereof, and Uses Thereof

WITNESS my hand this 25 day of April, Nineteen Hundred and Ninety-Seven.

Witnesses:

1) [Signature]
Name:

2) [Signature]
Name:

Helsinki University
Licensing, Ltd.

By: [Signature]
Heikki Lampi
President



POWER OF ATTORNEY

The Ludwig Institute for Cancer Research hereby appoints:

Alvin D. Shulman (19,412)
Owen J. Murray (22,111)
Allen H. Gerstein (22,218)
Nate F. Scarpelli (22,320)
Edward M. O'Toole (22,477)
Michael F. Borun (25,447)
Trevor B. Joike (25,542)

Timothy J. Vezou (26,348)
Carl E. Moore, Jr. (26,487)
Richard H. Anderson (26,526)
Patrick D. Ertel (26,877)
James P. Zeller (28,491)
William E. McCracken (30,195)
David A. Gass (38,153)

Richard A. Schnurr (30,890)
Anthony Nimmo (30,920)
Christine A. Dudzik (31,245)
Kevin D. Hogg (31,839)
Jeffrey S. Sharp (31,879)
Martin J. Hirsch (32,237)

James J. Napoli (32,361)
Richard M. La Bargo (32,254)
Karl A. Vick (33,288)
Douglass C. Hochstetler (33,710)
Cynthia L. Schaller (34,245)
Robert M. Gerstein (34,824)

as its attorneys, with full powers of substitution and revocation, to act on its behalf before the U.S. Patent and Trademark Office in connection with the following applications filed by Kari Alitalo et al. of which it is an assignee:

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>	<u>Assignment Reel & Frame #</u>
08/510,133	01/Aug/95	Receptor Ligand	8378/0566
08/585,895	12/Jan/96	Receptor Ligand	8145/0829
08/601,132	14/Feb/96	Antibodies Reactive with VEGF-C, a Ligand for the Flt4 receptor Tyrosine Kinase (VEGFR-3)	8129/0688
08/671,573	28/Jun/96	Receptor Ligand VEGF-C	8161/0909

Please continue to send correspondence to:

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
United States of America
(312) 474-6300

Ludwig Institute for Cancer Research
1345 Avenue of the Americas
New York, New York 10105

(Date) 26-01-98

By: 

Name: A. Munro

Title: ASSOCIATE DIRECTOR



POWER OF ATTORNEY

Helsinki University Licensing, Ltd., hereby appoints:

Alvin D. Shulman (19,412)
Owen J. Murray (22,111)
Allen H. Gerstein (22,218)
Nate F. Scarpelli (22,320)
Edward M. O'Toole (22,477)
Michael F. Borun (25,447)
Trevor B. Joike (25,542)

Timothy J. Vezau (26,348)
Carl E. Moore, Jr. (26,487)
Richard H. Anderson (26,526)
Patrick D. Ertel (26,877)
James P. Zeller (28,491)
William E. McCracken (30,195)
David A. Gass (38,153)

Richard A. Schnurr (30,890)
Anthony Nimmo (30,920)
Christine A. Dudzik (31,245)
Kevin D. Hogg (31,839)
Jeffrey S. Sharp (31,879)
Martin J. Hirsch (32,237)

James J. Napoli (32,361)
Richard M. La Barge (32,254)
Karl A. Vick (33,288)
Douglass C. Hochstetler (33,710)
Cynthia L. Schaller (34,245)
Robert M. Gerstein (34,824)

as its attorneys, with full powers of substitution and revocation, to act on its behalf before the U.S. Patent and Trademark Office in connection with the following applications filed by Kari Alitalo et al. of which it is an assignee:

Application No.	Filing Date	Title	Assignment Reel & Frame #
08/510,133	01/Aug/95	Receptor Ligand	8378/0566
08/585,895	12/Jan/96	Receptor Ligand	8145/0829
08/601,132	14/Feb/96	Antibodies Reactive with VEGF-C, a Ligand for the Flt4 Receptor Tyrosine Kinase (VEGFR-3)	8129/0688
08/671,573	28/Jun/96	Receptor Ligand VEGF-C	8161/0909

Please continue to send correspondence to:

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
United States of America
(312) 474-6300

Helsinki University Licensing, Ltd.
Viikinkaari 8 A
FIN-00710 Helsinki
FINLAND

(Date) 28th of June 1998

By: (Signature)
Name: Heikki Lampi
Title: President

Please enter the power of attorney documents into the file for the above-identified patent application.

Respectfully submitted,
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 
David A. Gass
Registration No. 38,153

Date: Feb 24, 1998



GAU-1801
1652

PATENT #2
28967/33072
03/12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No. 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

I hereby certify that this paper is
being deposited with the United
States Postal Service as first class
mail, postage prepaid, in an
envelope addressed to: Assistant
Commissioner for Patents,
Washington, D.C. 20231, on this
date:

Dated: Feb 24, 1998

David A. Gass
David A. Gass

TRANSMITTAL OF POWERS OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are power of attorney documents executed by the
two assignees of the above-identified patent application: Helsinki University
Licensing, Ltd., and The Ludwig Institute for Cancer Research.

The above-identified application was assigned by the inventors to
Helsinki University Licensing, Ltd., (HUL) in an assignment recorded at Reel
8145, Frame 0829.

HUL assigned a 50% interest in the application to The Ludwig Institute
for Cancer Research, as evidenced by the attached assignment document
which has been submitted for recordation.



**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
-----------------	-------------	----------------------	---------------------

08/595,895 01/12/96 ALITALO

K 28113/33072

EXAMINER

HM11/0324

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

BROWN, K

ART UNIT

PAPER NUMBER

1646

DATE MAILED:

03/24/98

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.

08/585,895

Applicant(s)

Alitalo et al.

Examiner

Brown

Group Art Unit

1646

—The MAILING DATE of this communication appears on the cover sheet beneath the correspondence address—

Period for Response

A SHORTENED STATUTORY PERIOD FOR RESPONSE IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a response be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for response specified above is less than thirty (30) days, a response within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for response is specified above, such period shall, by default, expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to respond within the set or extended period for response will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

- ☒ Responsive to communication(s) filed on 12/1/97
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

- ☒ Claim(s) 1, 3-5, 7, 11, 18-38 is/are pending in the application.
- Of the above claim(s) _____ is/are withdrawn from consideration.
- ☒ Claim(s) 33-36 is/are allowed.
- ☒ Claim(s) 1, 3-5, 7, 11, 18-32, 37-38 is/are rejected.
- ☐ Claim(s) _____ is/are objected to.
- ☐ Claim(s) _____ are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119 (a)-(d)

- ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some ☐ None of the CERTIFIED copies of the priority documents have been received.
- ☐ received in Application No. (Series Code/Serial Number) _____
- ☐ received in this national stage application from the International Bureau (PCT Rule 1.7.2(a)).

*Certified copies not received: _____

Attachment(s)

- ☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____
- ☒ Notice of References Cited, PTO-892
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Interview Summary, PTO-413
- ☐ Notice of Informal Patent Application, PTO-152
- ☐ Other _____

Office Action Summary

Serial Number: 08/585,895

Page 2

Art Unit: 1646

DETAILED ACTION

1. The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1646.

Response to Amendment

2. Claims 2, 6, 8-10 and 12-17 have been cancelled, and claims 26-38 have been added.

Therefore, claims 1, 3-5, 7, 11 and 18-38 are instantly examined.

3. The following rejections are withdrawn upon reconsideration and Applicant's amendments: The rejection of claims 1 and 18-25 under 35 USC 112, first paragraph, and the rejection of claims 3-5, 11 and 18-25 under 35 USC 112, second paragraph.

4. The declaration under 37 CFR 1.132 filed 1 December 1997 is insufficient to overcome the rejection of claims 7 and 37 based upon 35 USC 112, first paragraph, as set forth in this Office action in ¶10 below for the following reasons: Although the declaration of Alitalo at ¶5 states that complete sequencing of the cDNA insert contains the sequence of SEQ ID NO:44, the declaration does not state that the cDNA insert is derived from ATCC Deposit No. 97231. The declaration also does not state what is the relationship of the 1997 base pair cDNA to the "approximately 2.1 kb insert" of the pFLT4-L clone. If the "approximately 2.1 kb insert" of ATCC Deposit No. 97231 is what was sequenced and shown to be 1997 base pairs and have the sequence of SEQ ID NO:44, then this should be made clear. In addition, Applicant must state or declare that all restrictions regarding the availability of the deposited material must be irrevocably

Serial Number: 08/585,895

Page 3

Art Unit: 1646

removed upon the granting of the patent (see Paper No. 17, page 4, and below in ¶9-11 of this Office action).

5. The declaration is also insufficient to obviate the rejection of claims 1, 18, 23-31, 37 and 38 under 35 USC 112, first paragraph, as set forth in ¶12 of this Office action for the following reasons: Although the declaration of Alitalo demonstrates at ¶7-18 that fragments comprising portions of SEQ ID NO:33 bind to the Flt4 receptor and activate tyrosine phosphorylation, and that polynucleotides which hybridize to SEQ ID NO:32 encode VEGF-C polypeptides which bind to the Flt4 receptor and activate tyrosine phosphorylation, the showing is not commensurate in scope with the claims, which recite any protein which binds to Flt4 receptor, for those reasons provided in ¶12 of this Office action.

Oath/Declaration

6. The requirement for a new declaration is withdrawn in view of Applicant's second declaration, filed 12 August 1996.

Drawings

7. The proposed drawing correction and/or the proposed substitute sheets of drawings, filed on 1 December 1997 have been approved.

8. The wish to defer formal corrections of the drawings and the petition for photographs is acknowledged.

Art Unit: 1646

Sequence Rules

9. The submission of a new Sequence Listing and CRF containing the sequences of Figures 9B and 10 is acknowledged, and the requirement to comply with Sequence Rules is withdrawn.

Specification

10. The amendment filed 1 December 1997 is objected to under 35 U.S.C. 132 because it introduces new matter into the disclosure. 35 U.S.C. 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows: SEQ ID NOS: 44 and 45.

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97231 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO:44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO:44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "The approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO:44." It is further noted that the nucleotide sequence of the plasmid is not SEQ ID NO:45, as stated in the added sentence. SEQ ID NO:45 is a translated open reading frame of the nucleotide sequence of SEQ ID NO:44, as stated by Dr. Kari Alitalo (¶5). In addition, Applicant states that ATCC Deposit No. 97231 has been deposited under the terms of the Budapest Treaty; however,

Serial Number: 08/585,895

Page 5

Art Unit: 1646

Applicant must also state that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent (37 CFR 1.808), as discussed in Paper No. 17, page 4. If this statement is made, the objection to the specification will be withdrawn.

Claim Rejections - 35 USC § 112

11. Claims 7 and 37 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

This rejection is maintained for reasons of record set forth in Paper No. 17, pages 4-5.

12. Applicant's arguments filed 1 December 1997 have been fully considered but they are not persuasive.

Applicant argues that ATCC Deposit No. 97231 has been deposited under the terms of the Budapest Treaty; however, Applicant must also state that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent (37 CFR 1.808), as discussed in Paper No. 17, page 4. If this statement is made, the rejection will be withdrawn.

13. Claims 1, 18, 23-31, 37 and 38 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for while being enabling for a polynucleotide which encodes a polypeptide comprising a portion of SEQ ID NO:33 sufficient to bind to the Flt4

Art Unit: 1646

receptor tyrosine kinase and stimulate tyrosine phosphorylation of the Flt4 receptor, does not reasonably provide enablement for a polynucleotide which encodes polypeptide which is defined only by its binding to the Flt4 receptor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Claims 1, 18, 23-31, 37 and 38 are not commensurate in scope with the specification with respect to the recitation in claims 1, 26-29 and 37 of a polynucleotide which hybridizes to SEQ ID NO:32 and which encodes a polypeptide which binds to the Flt4 receptor or in claims 18, 23-25, 30-31 and 38 of a polynucleotide which encodes a polypeptide comprising a portion of SEQ ID NO:33 which binds to the Flt4 receptor. One skilled in the art could use the guidance in the specification to isolate polynucleotides which hybridize to SEQ ID NO:32 and test them for Flt4 receptor binding and tyrosine phosphorylation activity. Similarly, one skilled in the art could use the guidance in the specification to isolate polynucleotide which encode polypeptides which comprise portions of SEQ ID NOS:33 and test them for Flt4 receptor binding and tyrosine phosphorylation activity. However, claims 1, 18, 23-31, 37 and 38 currently recite only a polynucleotide which encodes a polypeptide which binds the Flt4 receptor, and thus these claims encompass polypeptides which bind to the Flt4 receptor under any condition and which have no biological activity. It is well-known in the art that a growth factor ligand must not only bind to its receptor but also be able to induce some biochemical signal, such as phosphorylation, in order to have a biological effect (Borg, p. 981, col. 2). Neither the specification nor the prior art teaches

Serial Number: 08/585,895

Page 7

Art Unit: 1646

one skilled in the art how to use a polypeptide which binds to the Flt4 receptor and which does not stimulate the tyrosine phosphorylation activity of the receptor. Furthermore, given the large number of different biochemical pathways which may or may not be activated by polypeptide binding, and given the lack of guidance in the specification, one skilled in the art would not know which of the many biochemical signaling pathways, other than tyrosine phosphorylation, to examine in order to determine whether a polynucleotide which encodes a polypeptide comprising a portion of SEQ ID NO:33 might activate. Similarly, one skilled in the art would not know how to use a polynucleotide which hybridized to SEQ ID NO:32 and which encoded a polypeptide which bound to Flt4 receptor but which did not activate tyrosine phosphorylation. Absent such guidance, one skilled in the art would not know how to use a polynucleotide which encoded a polypeptide which binds to the Flt4 receptor but which does not stimulate tyrosine phosphorylation. Therefore, it would require undue experimentation to practice this invention as claimed.

This rejection could be overcome by amending the claims to recite that the encoded polypeptide not only binds to the human Flt4 receptor but stimulates tyrosine phosphorylation of the Flt4 receptor tyrosine kinase, such as is recited in claim 19.

14. Applicant's arguments regarding the previous rejection under 35 USC 112, first paragraph, have not been addressed as the previous rejection of the claims has been withdrawn.

Art Unit: 1646

15. Claims 1, 3-5, 7, 11, 18-30, 32 and 37-38 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

16. Claims 1, 3-5, 7, 26-29 and 37 are indefinite with respect to the term "a domain defined by eight conserved cysteine residues." It is unclear to what the eight residues are conserved. It is also unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter. Furthermore, these claims are indefinite with respect to the term "having homology to vascular endothelial growth factor." It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF (see Reeck et al. Cell, 50, 667).

17. Claims 1, 3-5, 7, 11, 18-30, 32 and 37-38 are indefinite because it is unclear what is a domain encompassed by "cysteine motifs of a Balbiani ring 3 protein." Since the BR3P domain is not defined in the specification, one cannot determine what a BR3P domain is. Furthermore, it is unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter.

18. Claims 1, 26-29 and 37 are indefinite with respect to the term "high affinity." The term "high affinity" is relative, and it is not clear how strongly a protein must bind to the Flt4 receptor in order for it to be considered "high affinity." It is suggested that the claims be amended to recite a particular range of K_d .

Art Unit: 1646

19. Claims 1, 3-5, 7, 26-30 and 37 are indefinite with respect to the term "including." It is unclear whether "including" is equivalent to the open language "comprising" or to the closed language "consisting of."
20. Claims 3, 5, 18, 24-25 and 30-31 are indefinite because the term "said polynucleotide" lacks antecedent basis.
21. Claim 30 is indefinite with respect to the term "VEGF-homologous portion." It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF (see Reeck et al. Cell, 50, 667).
22. Claim 32 is indefinite with respect to an amino acid sequence "corresponding to" another amino acid sequence. It is unclear whether "corresponding to" means that the amino acid sequence is identical or not.
23. Applicant's arguments regarding the previous rejection under 35 USC 112, second paragraph, have not been addressed as the previous rejection of the claims has been withdrawn.
24. Applicant is correct that the publication date of Reference B1 does not antedate the effective filing date of the instant application, and thus does not anticipate or render obvious the claimed invention because it is not available as prior art.
25. Applicant's arguments regarding Reference B1 are noted; however, since no rejection has been made over this patent, these arguments are not addressed.

Conclusion

26. Claims 33-36 are allowed.

Serial Number: 08/585,895

Page 10

Art Unit: 1646

27. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Brown whose telephone number is (703) 308-3667. The examiner can normally be reached on Mondays through Thursdays and on alternate Fridays from 8:30 to 6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Stephen Walsh, can be reached on (703) 308-2957.

Official papers filed by fax should be directed to (703) 305-4242. Faxed draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

KEB

KEB

20 March 1998

Stephen Walsh
STEPHEN WALSH
SUPERVISORY PATENT EXAMINER
GROUP 1800

Notice of References Cited				Application No. 081585,895		Applicant(s) Alitelco et al.	
				Examiner Brown		Group Art Unit 1646	
Page 1 of 1							
U.S. PATENT DOCUMENTS							
*	DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS		
A							
B							
C							
D							
E							
F							
G							
H							
I							
J							
K							
L							
M							
FOREIGN PATENT DOCUMENTS							
*	DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUBCLASS	
N							
O							
P							
Q							
R							
S							
T							
NON-PATENT DOCUMENTS							
*	DOCUMENT (Including Author, Title, Source, and Pertinent Pages)						DATE
U	Roeck et al. Cell 50,667.						1987.
V							
W							
X							

Patent Clerk
PATENT

28967/33072

7/24/98

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

In re Application of:)	I hereby certify that this paper is being
)	deposited with the United States Postal
Alitalo et al.)	Service as first class mail, postage
)	prepaid, in an envelope addressed to:
Serial No.: 08/585,895)	Assistant Commissioner for Patents
)	Washington, D.C. 20231, on this date:
Filed: January 12, 1996)	
)	Dated: <u>Nov. 26, 1997</u>
Title: RECEPTOR LIGAND)	
)	
Art Unit: 1801)	
)	<i>David A. Gass</i>
Examiner: Lathrop, B.)	David A. Gass
)	Registration No. 38,153

DECLARATION OF BIOLOGICAL CULTURE DEPOSIT
IN COMPLIANCE WITH BUDAPEST TREATY REQUIREMENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, the undersigned, declare that:

1. I am an inventor of the subject matter of the above-identified patent application.

2. The plasmid designated FLT4-L, described in the specification of the above-identified application at pages 28-29 (and elsewhere), was deposited on 24 July 1995 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, under the terms of the Budapest Treaty. This plasmid was assigned ATCC accession number 97231. A copy of the ATCC deposit receipt, confirming viability of the deposit, is attached hereto.

3. With respect to the permanence of the deposit, the ATCC is an official depository in accordance with the Budapest Treaty for the above-deposited material, and I affirm that, should the plasmid identified in paragraph 2 mutate, become non-viable, or be inadvertently destroyed, I will replace it for at least thirty (30) years from the date of the original deposit, or for at least five (5) years from the date of the most recent request for release of a sample, or for the enforceable life of any patent issued on the above-mentioned application, whichever period is longest.

4. With respect to availability of the plasmid identified in paragraph 2, I affirm that the deposit has been made under conditions of assurance of (a) ready accessibility thereto by the public if an enforceable patent is granted whereby all restrictions to the availability to the public of the culture so deposited will be irrevocably removed upon the granting of the patent [MPEP §608.01 (p)], and (b) access to the deposit will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122.

5. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

November 20, 1997
Date

Kari Alitalo
Kari Alitalo



American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301) 231-5520 Telex: 898-655 ATCCNORTH • FAX: 301-770-2587

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

University of Helsinki
Attention: Kari Alitalo
Molecular/Cancer Biology Laboratory
P.O. Box 21 (Haartmaninkatu 3)
SF-00014, HELSINKI, FINLAND

Deposited on Behalf of: Kari Alitalo and Vladimir Joukov

Identification Reference by Depositor:

ATCC Designation

Plasmid, FLT4-L

97231

The deposit was accompanied by: ☐ a scientific description ☐ a proposed taxonomic description indicated above.

The deposit was received July 24, 1995 by this International Depository Authority and has been accepted.

AT YOUR REQUEST:

☒ We will not inform you of requests for the strain.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested August 1, 1995. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Annette L. Bade, Director, Patent Depository

Date: August 9, 1995

cc: Thomas C. Meyers



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Alitalo *et al.*

Serial No. 08/585,895

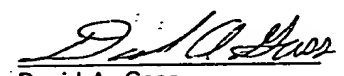
Filed: January 12, 1996

For: RECEPTOR LIGAND

Art Unit: 1646

Examiner: Saoud

) I hereby certify that this paper is
) being deposited with the United
) States Postal Service as first class
) mail, postage prepaid, in an
) envelope addressed to: Assistant
) Commissioner for Patents,
) Washington, D.C. 20231, on this
) date: July 23, 1998

) 
) David A. Gass
) Attorney for Applicants
)

AMENDMENT AND REPLY PURSUANT TO 37 C.F.R. § 1.111

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In an official action mailed March 24, 1998, the U.S. Patent and Trademark Office (the Patent Office) allowed claims 33-36, but rejected claims 1, 3-5, 7, 11, 18-32, and 37-38 variously under 35 U.S.C. §§ 112, first and second paragraphs. The Patent Office also objected to an amendment under § 132, alleging that the amendment introduced new matter. The Applicants respectfully request reconsideration in light of the following amendments and remarks. This amendment has been timely filed with a petition and fee for one month extension of time, extending the shortened statutory period to July 24, 1998.

AMENDMENTS

In the specification:

Please amend the specification as set forth below:

Please delete the amendment to the priority claim at page 1, line 3, filed on August 12, 1996, and substitute therefor the following updated priority claim: -- This application ^{AND} is also a continuation-in-part of U.S. Patent Application Serial No. 08/340,011, filed November 14, 1994, now U.S. Patent No. 5,776,755. --

At page 5, line 21, delete "SEQ ID NO: 2" and substitute therefor --SEQ ID NO: 33--.

At page 5, line 31, delete "polypeptide" and substitute therefor --polypeptides--.

Please cancel the amendment to page 29, line 1, of the specification made on November 26, 1996, and substitute therefor the following amendment at the same location: -- The approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a nucleotide sequence that includes the 1997 nucleotides of sequence set forth in SEQ ID NO: 44. The nucleotide sequence set forth in SEQ ID NO: 44 encodes the 419 residue amino acid sequence set forth in SEQ ID NO: 45. --

At page 29, line 3, delete "this reading frame" and substitute therefor the reading frame specified in SEQ ID NOs: 32-33.

At page 31, line 20, after "ORF" please insert specified in SEQ ID NOs: 32 and 33.

In the claims:

Please amend claims 1, 3-5, 7, 18-19, 26-33 and 36-37; and add new claims 39-44 as shown below:

D5

1. (Three times amended) A host cell transformed or transfected with a polynucleotide [encoding a polypeptide that is capable of binding with high affinity to the extracellular domain of human Flt4 receptor tyrosine kinase], wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses a polypeptide encoded by said polynucleotide, [said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)]

wherein said polypeptide includes a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).

wherein said polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase.

D6

3. (Three times amended) A host cell transformed or transfected with a [nucleic acid encoding] polynucleotide comprising a nucleotide sequence that encodes [a polypeptide having] the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, [said polypeptide including a domain defined by eight conserved

cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)] said polypeptide including a contiguous portion of SEQ ID NO: 33 that is sufficient to bind to the extracellular domain of human Flt4 receptor tyrosine kinase (Flt4EC).

wherein said contiguous portion includes eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).

wherein said polypeptide lacks any portion of SEQ ID NO: 33 that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and wherein said polypeptide is capable of binding to Flt4EC.

D6 4. (Twice amended) A host cell according to claim 3 wherein said [nucleic acid] nucleotide sequence comprises nucleotides 37 to 1086 of the sequence shown in SEQ ID NO: 32.

5. (Three times amended) A host cell according to claim 3 wherein said polynucleotide is a vector comprising [a nucleic acid] an expression control sequence operatively linked to the nucleotide sequence that encodes [a polypeptide having] the amino acid sequence shown in SEQ ID NO: 33.

D7 7. (Twice amended) A host cell comprising the insert of plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses and secretes a polypeptide encoded by said insert. [plasmid, said polypeptide including a domain defined by eight conserved cysteines having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)]

wherein said secreted polypeptide binds to human Flt4 receptor tyrosine kinase and includes a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human

platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and

wherein said secreted polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P).

18. (Twice amended) A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to [an] human Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid [polynucleotide] lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

19. (Amended) A purified and isolated nucleic acid according to claim 18 wherein said polypeptide is capable of stimulating tyrosine phosphorylation of human Flt4 receptor tyrosine kinase.

26. (Amended) A host cell according to claim 1 that expresses a naturally occurring [VEGF-C] Flt4 ligand protein encoded by said polynucleotide.

27. (Amended) A host cell according to claim 1 that expresses a human [VEGF-C] Flt4 ligand protein encoded by said polynucleotide.

28. (Amended) A host cell according to claim [27] 1, wherein said host cell expresses said polynucleotide and produces a [mature] human [VEGF-C] protein that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase, said protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. (Amended) A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to [a nucleotide] sequence that encodes said polypeptide.

D10 30. (Amended) A [polynucleotide] nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes [a VEGF-homologous portion] eight cysteines of SEQ ID NO: 33 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and excludes the carboxyl terminal portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

31. (Amended) A [polynucleotide] nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. (Amended) A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence [corresponding] identical to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. (Amended) A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence consisting of a continuous portion of the sequence shown in SEQ ID NO: 33, said continuous portion

D10 commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

36. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

D11 growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell, wherein said polypeptide is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating phosphorylation of Flt4 receptor tyrosine kinase.

37. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B). [conserved cysteines and having homology to vascular endothelial growth factor (VEGF)] and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

– 39. A method according to claim 38 wherein said host cell is a mammalian host cell that secretes said polypeptide and wherein said isolating step comprises isolating said polypeptide from said growth medium.

40. A eukaryotic host cell according to claim 1 or 3 that secretes said polypeptide.

D12 41. A nucleic acid according to claim 30 wherein said continuous portion has amino acid 1 of SEQ ID NO: 33 as its amino terminus.

42. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase, wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses and secretes a polypeptide encoded by said polynucleotide, and

wherein said polypeptide binds the extracellular domain of human Flt4 receptor tyrosine kinase and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

43. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds human Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a

continuous portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

D12 44. A purified and isolated nucleic acid according to claim 43 wherein said nucleic acid lacks a nucleotide sequence that encodes the amino terminal portion of the amino acid sequence shown in SEQ ID NO: 33 that precedes residue 1.

REMARKS

I. History of claims and explanation of amendments.

A. Prosecution History

The application as filed contained 16 claims.

In an official communication dated November 25, 1996, claims 1-16 were subjected to a restriction requirement. In an Amendment and Election in Response to Restriction Requirement filed on January 24, 1997, the Applicants: elected claims directed to nucleic acids, vectors, and host cells; canceled claims 2, 8-10, 12, and 14-16; amended claims 1, 3, 5, 11, and 13; and added claims 17-25.

In an Office action dated May 28, 1997, claims 1-3, 7, 11, 13, 17-25 were rejected. In a responsive amendment dated November 26, 1997, the Applicants canceled claims 6, 13, and 17; amended claims 1, 3-5, 7, 11, 18, and 20; and added new claims 26-38. Thus, claims 1, 3-5, 7, 11 and 18-38 were pending at the time the outstanding Office action was issued. In the outstanding Office action, claims 33-36 have been allowed, but claims 1, 3-5, 7, 11, 18-32, and 37-38 were rejected.

In the present amendment, the Applicants amend claims 1, 3-5, 7, 18-19, 26-33, and 36-37; and add new claims 39-44. A copy of the claims, in their amended forms, is appended hereto for the Examiner's convenience.

nucleotides 37 to 1086 represent the portion of SEQ ID NO: 32 that encodes the amino acid sequence specified in SEQ ID NO: 33.

The amendment to claim 5 to recite a vector comprising "an expression control sequence operatively linked" to a coding sequence finds support throughout the application, including at page 6, lines 28-30.

The amendment to claim 7 to specify that the host cell "secretes" the encoded polypeptide, and to specify that the secreted polypeptide binds to Flt4, is found throughout the application. For example, Example 11 (p. 28) of the application describes the expression and secretion into the cell culture medium of a polypeptide encoded by the insert of the deposited plasmid. The polypeptide bound Flt4 and stimulated Flt4 phosphorylation. New claims 39-40 are likewise supported by way of example (see Examples 6, 11, and 13, for example, teaching the use of eukaryotic/mammalian expression vectors and cell lines to express VEGF-C).

Claims 18 and 19 have been amended to recite "human" (i.e., "human Flt4"). This amendment is not intended to imply that polypeptides of the invention which bind to human Flt4 would not also bind to Flt4 proteins of other animals. Claim 18 also has been amended to recite "nucleic acid" instead of "polynucleotide." This amendment is not intended to alter the scope of the claim, but merely to use a term that has *ipsis verbis* antecedent basis in the preamble.

Claims 26 and 27 have been amended to recite "Flt4 ligand" instead of "VEGF-C." This amendment is not intended to diminish the scope of the claims, since VEGF-C is the name ascribed to an Flt4 ligand of the invention. See, e.g., specification at page 5, lines 17-19. Similarly, claim 28 has been amended to recite a human protein "that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase," instead of reciting human "VEGF-C."

Claim 28 also has been amended to delete the term "mature," which is believed to be unnecessary to define the invention, especially in view of the binding and molecular weight limitations of the claim.

The amendment to allowed claim 33 to recite "said polypeptide *having an amino acid sequence* consisting of a continuous portion of the sequence shown in SEQ ID NO: 33" is formal in nature and not intended to diminish the scope of the claim.

Likewise, the amendment to allowed claim 36 merely makes the language of the final step of the claim more closely parallel the language of the preamble. This amendment is formal in nature and not intended to diminish the scope of the claim.

New claim 41 finds support throughout the application as originally filed, including at page 23, lines 1-10, and claim 10 as originally filed.

New claim 42 is directed to a host cell transformed or transfected with a polynucleotide. The hybridization conditions recited in claim 42 are identical to those recited in claim 1 and find support, e.g., in Example 10 at page 27, lines 10-14. The recitation in claim 42 that the host cell "secretes" the expressed polypeptide finds support, e.g., in Example 11 (p. 28). The size and binding characteristics that are recited in new claim 42 find support throughout the application as originally filed, including in Example 5 and in original claims 8 and 9.

New claims 43-44 find support throughout the application as originally filed, including at page 6, lines 16-20. The specified terminal amino acids in claims 43-44 find support, e.g., at page 5, lines 27-34, and are the same terminal residues specified in allowed claim 33.

II. The rejection of claims 7 and 37 under 35 U.S.C. § 112, first paragraph, was improper, and should be withdrawn.

Paragraphs 4 and 10-12 pertain to a rejection of claims 7 and 37 under 35 U.S.C. § 112, first paragraph. The Patent Office indicates that it will withdraw the rejection if an appropriate statement is filed lifting all restrictions on the availability of a deposited plasmid, consistent with Budapest treaty. *The Applicants filed such a statement with their amendment dated November 26,*

1997. A copy of the statement is filed herewith. Thus, the objection to the specification and rejection should now be withdrawn.

III. The objection that a previous amendment introduced new matter should be withdrawn.

In paragraphs 4 and 10 of the Office action, the Patent Office objects to an amendment to introduce SEQ ID NO: 44 and 45 into the application, alleging that the amendment introduces new matter:

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97321 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO: 44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO: 44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "The approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO: 44." It is further noted that the nucleotide sequence of the plasmid is not SEQ ID NO: 45, as stated in the added sentence. SEQ ID NO: 45 is a translated open reading frame of the nucleotide sequence of SEQ ID NO: 44

(Office action at p. 4; see also p. 2.)

The Applicants respectfully traverse.

The allegation that the previously-filed Rule 132 Alitalo declaration fails to state that the cDNA insert was derived from ATCC Deposit No. 97231 is incorrect. Paragraph 4 of the declaration identifies the plasmid by its ATCC accession number and paragraph 5 states, "Attached hereto as Exhibit B is a 1997 nucleotide sequence of the cDNA that was deposited with the ATCC. Exhibit B also depicts the deduced 419 amino acid open reading frame. These sequences have been added to the patent application as SEQ ID NOs: 44 and 45." Thus, the amendment to add SEQ ID NOs: 44-45 to the application had sufficient corroboration.

Notwithstanding the foregoing, the Applicants have adopted all of the Patent Office's suggestions to overcome the new matter objection. The Applicants have amended the application at page 29 to explain the relationship between the approximately 2.1 kb insert and the 1997 base pair sequence; the Applicants have clarified the DNA/encoded protein relationship between SEQ ID NO: 44 and 45; and the Applicants have filed herewith another declaration from Dr. Alitalo confirming that SEQ ID NOs: 44 and 45 represent nucleotide and deduced amino acid sequences of the deposited plasmid. Accordingly, the new matter objection should now be withdrawn.

IV. The rejection of claims 1, 18, 23-31, and 37-38 under §112, first paragraph, should be withdrawn.

In paragraphs 5 and 13 of the outstanding Office action, the Patent Office rejected claims 1, 18, 23-31, and 37-38 under §112, first paragraph, alleging that the specification does not reasonably enable the full scope of these claims. As its basis for rejection, the Patent Office alleges that neither the application nor the prior art enables one skilled in the art to use a polypeptide which binds to the Flt4 receptor and which does NOT stimulate tyrosine phosphorylation activity of the receptor. (Office action at pp. 5-7.) The Applicants respectfully traverse.

The present patent application teaches uses for polypeptides of the invention that bind, but fail to activate, the Flt4 receptor. For example, at page 7, lines 8-15, the application teaches that Flt4 ligand polypeptides of the invention can be labeled and used to identify their corresponding receptor *in situ*. Such labeled ligands can be used as detection or imaging agents, analogous to anti-Flt4 antibodies, to detect and/or image lymphatic vessels and high endothelial venules that express the Flt4 receptor on their surface. Such imaging/detection uses include uses for analyzing histochemical tissue sections. Those skilled in the art understand that the activity of binding to the extracellular domain of Flt4 is all that is required to make polypeptides effective for such uses. Stated differently, imaging a receptor with a labeled binding

agent does not require the labeled binding agent to activate the receptor. Such uses were discussed in an interview of March 24, 1998, in a related application (USSN 08/671,573), at which time Examiner Brown acknowledged that she had not considered such uses when entering the rejection. A similar rejection in the related application has now been withdrawn by the Patent Office.

The application also teaches that peptides which block the Flt4 receptor are useful as inhibitors to control endothelial cell proliferation and lymphangiomas. (See page 7, line 32, to page 8, line 2.) Persons skilled in the art understand that polypeptides that bind to the receptor but fail to activate the receptor can serve as competitive inhibitors. Thus, the application provides this additional use for polypeptides that bind Flt4 but fail to stimulate tyrosine phosphorylation of the receptor.

Because the present application teaches those skilled in the art "how to use a polypeptide which binds to the Flt4 receptor but which does not stimulate tyrosine phosphorylation," the Patent Office's basis for rejection is unfounded. Accordingly, the rejection of claims 1, 18, 23-31, and 37-38 under §112, first paragraph, should be withdrawn.

- V. The Patent Office's rejections of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 under 35 U.S.C. §112, second paragraph, should be withdrawn.

In paragraphs 15-22 of the Office action, the Patent Office rejected claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 under 35 U.S.C. §112, second paragraph, alleging several bases why these claims were indefinite. The Applicants traverse-in-part and amend-in-part.

- A. The rejection of claims 1, 3-5, 7, 26-29, and 37 relating to the term "a domain defined by eight conserved cysteine residues" should be withdrawn.

In paragraph 16 of the Office action, the Patent Office rejected claims 1, 3-5, 7, 26-29, and 37, alleging that the term "a domain defined by eight conserved cysteine residues" was indefinite. The Applicants traverse-in-part and amend-in-part.

1. It is clear to what the eight residues are conserved.

As its first rationale for rejection, the Patent Office asserted, "It is unclear to what the eight residues are conserved." (Office action at p. 8.) The Applicants' amendments render this rationale moot. For example, claim 1 has been amended to recite, "wherein said polypeptide includes a domain defined by eight cysteine residues *that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).*" Claims 3, 7, and 37 have been amended similarly. The eight conserved cysteines are readily apparent to scientists skilled in the art. (See the alignment of VEGF, PDGF-A, and PDGF-B in Fig. 10A of the patent application, with conserved cysteines at positions 103, 130, 136, 139, 140, 147, 184, and 186.) Since the claim now recites "to what the eight residues are conserved," the basis for rejection is rendered moot.

2. The minimum limits of the domain are clear.

As its second rationale for rejection, the Patent Office asserted, "It is also unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter." (Office action at p. 8.)

The basis for the rejection is contrary to the plain language of the claims. If a domain is "defined by eight conserved cysteines," then it clearly is not "defined by a different parameter." Thus, the plain language of the claims demonstrates that this basis for rejection is improper, and that the minimum included portion of the encoded polypeptide is defined with particularity. Accordingly, the rejection should be withdrawn.²

² Moreover, the Applicants' amendments to claim 3 render this basis for rejection moot with respect to claims 3-5, because claim 3 no longer recites "domain defined by." Instead, claim 3 recites a "contiguous portion of SEQ ID NO: 33" that "includes" the eight conserved cysteines.

3. The objection to the term "homology" is now moot.

As a third basis for rejection the Patent Office alleged, "these claims are indefinite with respect to the term 'having homology to vascular endothelial growth factor.' It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF." (Office action at p. 8.)

The Applicants respectfully submit that this phrase is clear and that "similarity" and "common evolutionary origin" are not incompatible concepts. However, solely to expedite allowance, the Applicants have deleted the allegedly indefinite term from claims 1, 3, 7, and 37, rendering this basis for rejection moot.

4. Conclusion

For the reasons set forth above, the rejection of claims 1, 3-5, 7, 26-29, and 37 should be withdrawn.

- B. The rejection of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 with respect to the term "cysteine motifs of a Balbiani ring 3 protein" should be withdrawn.

In paragraph 17 of the Office action, the Patent Office rejected claim 1, 3-5, 7, 11, 18-30, 32, and 37-38, alleging that the phrase "cysteine motifs of a Balbiani ring 3 protein" in claims 1, 3, 7, 18, 30, and 37 is indefinite. The Applicants respectfully traverse.

The Patent Office's first basis for rejection rests upon the two-part premise that "Since the BR3P domain is not defined in the specification, one cannot determine what a BR3P domain is." (Office action at p. 9.) Neither part of this premise is correct. The specification adequately defines the cysteine motifs of a Balbiani ring 3 protein (BR3P) at page 11, lines 16-25, citing two articles in the literature (both of record).³ The citation to literature in the art is

³ As discussed in paragraph 6 of the Rule 132 declaration of Dr. Alitalo dated November 26, 1997, BR3P cysteine motifs are quite distinctive in character (Cys-Xaa_n-Cys-Xaa-Cys-Xaa-Cys) and occur at least four times in the

more than adequate to describe that which is already known in the art. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986) (It is axiomatic that patent applications need not contain, and preferably omit, that which is well known in the art.).⁴

Moreover, even if the specification lacked the description at page 11, the fact remains that the characteristic BR3P cysteine motif was within the knowledge of those skilled in the art at the time of filing, such that one skilled in the art could determine whether or not a polypeptide contained a domain characterized by one or more BR3P cysteine motifs. See M.P.E.P. § 2164.08 ("Not everything necessary to practice the invention need be disclosed. In fact, what is well-known is best omitted.") Thus, the patent application contains sufficient definition of cysteine motifs of a BR3P protein for the reader skilled in the art. See, e.g., *In re Moore*, 169 U.S.P.Q. 236, 238 (CCPA 1971) (Claim language must not be analyzed in a vacuum, "but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary level of skill in the pertinent art.").

The Patent Office's second basis for rejection alleges that "it is unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter." (Office action at p. 8.) The Applicants respectfully submit that the plain language of the claims state that the claimed polypeptides lack portions *defined by the cysteines*, and that no reasonable alternative interpretation exists. For example, amended claim 1 recites, in

carboxy terminal portion of the VEGF-C precursor polypeptide (see, e.g., SEQ ID NO: 44, Cys residues at positions 280, 291, 293, 295; residues 304, 315, 317, 319; residues 328, 339, 341, and 343; and residues 347, 358, 360, and 362). The application depicts the VEGF-C precursor amino acid sequence, and the distinctive BR3P motifs in the carboxy-terminus would be readily apparent to the reader skilled in the art.

⁴ Notwithstanding the accepted practice of omitting that which is well known in the art, the Applicants will amend the specification to include an excerpt from the cited Dignam and Case article, if the Patent Office requests.

pertinent part, that the polypeptide "lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P)." There is no ambiguity to determining whether or not a protein's amino acid sequence includes or lacks one or more "Cys-Xaa_n-Cys-Xaa-Cys-Xaa-Cys" sequences.

For all of these reasons, the rejection of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 should be withdrawn.

- C. The rejection of claims 1, 26-29, and 37 with respect to the term "high affinity" has been rendered moot.

In paragraph 18 of the Office action, the Patent Office rejected claims 1 and claims 26-29, and 37 which depend therefrom, alleging that the claims were indefinite with respect to the term "high affinity" recited in claim 1: "The term 'high affinity' is relative, and it is not clear how strongly a protein must bind to the Flt4 receptor in order for it to be considered 'high affinity.' It is suggested that the claims be amended to recite a particular range of K_d ." (Office action at p. 8.)

The Applicants respectfully submit that the term "high affinity" is not indefinite to a person of ordinary skill in the art in view of the teachings of the application and the art to which the invention pertains. Notwithstanding this fact and solely to expedite allowance, the Applicants have amended claim 1 to delete the allegedly indefinite term, rendering this basis for rejection moot. The subject matter of the claim is adequately defined by the limitations that remain after this amendment.

- D. The rejection of claims 1, 3-5, 7, 26-30, and 37 with respect to the term "including" should be withdrawn.

In paragraph 19 of the outstanding Office action, the Patent Office rejected claims 1, 3-5, 7, 26-30, and 37, alleging that the term "including" was indefinite because "it is unclear whether 'including' is equivalent to the open language 'comprising' or the closed language 'consisting of.'" (Office action at p. 9.) The Applicants respectfully traverse. The term "including" is unequivocally interpreted as open claim language, synonymous with the term

"comprising." See M.P.E.P. §2111.03. Accordingly, this rejection should be withdrawn.

- E. The rejection of claims 3, 5, 18, 24-25, and 30-31 for lack of antecedent basis has been rendered moot.

In paragraph 20 of the outstanding Office action, the Patent Office rejected claims 3, 5, 18, 24-25, and 30-31, alleging that the term "said polynucleotide" as recited in the claims lacks antecedent basis. The Applicants have amended claim 3 to provide *ipsis verbis* antecedent basis for the term "said polynucleotide," thereby rendering the rejection moot with respect to claim 3 and also claim 5 which depends from claim 3.

The Applicants have amended claims 18 and 30-31 to recite "nucleic acid" instead of "polynucleotide." This substitution of terminology renders moot the rejection of claims 18, 24-25, and 30-31. The term "nucleic acid" in the amended claims has *ipsis verbis* antecedent basis support.

For these reasons, the rejection of claims 3, 5, 18, 24-25, and 30-31 has been rendered moot, and should be withdrawn.

- F. The rejection of claim 30 has been rendered moot.

In paragraph 21 of the Office action the Patent Office alleged, "Claim 30 is indefinite with respect to the term 'VEGF-homologous portion.' It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF" (Office action at p. 9.) The Applicants respectfully submit that this phrase is clear and that "similarity" and "common evolutionary origin" are not incompatible concepts. However, solely to expedite allowance, the Applicants have deleted the allegedly indefinite term, rendering this basis for rejection moot. Accordingly, the rejection of claim 30 should be withdrawn.

G. The rejection of claim 32 has been rendered moot.

In paragraph 22 of the Office action, the Patent Office alleged, "Claim 32 is indefinite with respect to an amino acid sequence 'corresponding to' another amino acid sequence. It is unclear whether 'corresponding to' means that the amino acid sequence is identical or not." (Office action at p. 9.) Solely to expedite allowance, the Applicants have substituted the term "identical to" for the term "corresponding to" in claim 32, rendering this rejection moot.

H. Conclusion.

For all of the foregoing reasons, the Patent Office's rejections of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 under 35 U.S.C. §112, second paragraph, should now be withdrawn.

VI. Status update relating to priority applications.

The 1994 priority application has now issued as U.S. Patent No. 5,776,712. The Applicants wish to apprise the Examiner that prosecution has been suspended in U.S.S.N. 08/510,133 because "A reference relevant to the examination of this application may soon become available."

VII. Summary

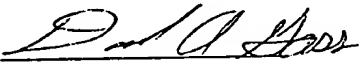
The Applicants respectfully request entry of the foregoing amendments and allowance of all of the pending claims in view of the foregoing remarks.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

Dated: July 23, 1998


David A. Gass
Registration No. 38,153

22. A purified and isolated nucleic acid according to claim 21 wherein said polypeptide comprises approximately 120 amino acids.

23. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

24. A vector comprising a nucleic acid according to claim 18.

25. A host cell transformed or transfected with a vector according to claim 24.

26. (Amended) A host cell according to claim 1 that expresses a naturally occurring Flt4 ligand protein encoded by said polynucleotide.

27. (Amended) A host cell according to claim 1 that expresses a human Flt4 ligand protein encoded by said polynucleotide.

28. (Amended) A host cell according to claim 1, wherein said host cell expresses said polynucleotide and produces a human protein that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase, said protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. (Amended) A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to sequence that encodes said polypeptide.

30. (Amended) A nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes eight cysteines of SEQ ID NO: 33 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and excludes the carboxyl terminal portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

31. (Amended) A nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. (Amended) A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence identical to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. (Amended) A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence consisting of a continuous portion of the sequence shown in SEQ ID NO: 33, said continuous portion commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

34. An expression construct comprising the polynucleotide according to claim 33 operatively linked to an expression control sequence.

35. A host cell transformed or transfected with the expression construct of claim 34.

36. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell, wherein said polypeptide is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating phosphorylation of Flt4 receptor tyrosine kinase.

37. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

38. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 25 under conditions which permit expression by said host cell of a polypeptide encoded by said nucleic acid that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

39. A method according to claim 38 wherein said host cell is a mammalian host cell that secretes said polypeptide and wherein said isolating step comprises isolating said polypeptide from said growth medium.

40. A eukaryotic host cell according to claim 1 or 3 that secretes said polypeptide.

41. A nucleic acid according to claim 30 wherein said continuous portion has amino acid 1 of SEQ ID NO: 33 as its amino terminus.

42. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase,

wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses and secretes a polypeptide encoded by said polynucleotide, and

wherein said polypeptide binds the extracellular domain of human Flt4 receptor tyrosine kinase and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

43. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds human Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a continuous portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

44. A purified and isolated nucleic acid according to claim 43 wherein said nucleic acid lacks a nucleotide sequence that encodes the amin terminal portion of the amino acid sequence shown in SEQ ID NO: 33 that precedes residue 1.



GAU 16
#2
PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)	Title: RECEPTOR LIGAND
Serial No: 08/585,895)	Group Art Unit: 1646
Filed: January 12, 1996)	Examiner: Saoud

**AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME**

RECEIVED
AUG 4 1998

**Assistant Commissioner for Patents
Washington, D.C. 20231**

**MAIL ROOM
SERVICE CENTER**

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111;
2. Declaration Under 37 C.F.R. § 1.132 of Dr. Kari Alitalo;
3. Declaration of Biological Culture Deposit in Compliance with Budapest Treaty Requirements;
4. Check in the amount of \$55.00 in payment of fee for extension of time; and
5. Check in the amount of \$159.00 in payment of fee for extra claims.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on July 23, 1998, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

☒ Small entity status has been established and is still effective.

2. **Extension of Time**

☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month		\$110.00	X	\$55.00
Two Months		\$400.00		\$200.00
Three Months		\$950.00		\$475.00
Four Months		\$1,510.00		\$755.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$55.00

☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$_____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$0

Extension Fee Due With This Request \$55.00

RECEIVED

AUG 4 1998

MATRIX CUSTOMER
SERVICE CENTER

3. Fee for Claims

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For	Present Extra		Rate	Additional Fee	Rate	Additional Fee
TOTAL	40	MINUS 33	7		X11 =	\$77	X22 =	\$
INDEP.	7	MINUS 5	2		X41 =	\$82	X82 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim					+ 135 =		+ 270 =	\$
TOTAL ADDITIONAL FEE						\$159	OR	\$

4. Method of Payment of Fees

☒ Attached are checks in the amount of \$55.00 and \$159.00.

☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

5. Deposit Account and Refund Authorization


The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:


David A. Gass
Reg. No: 38,153

July 23, 1998



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.) Title: RECEPTOR LIGAND
Serial No: 08/585,895) Group Art Unit: 1646
Filed: January 12, 1996) Examiner: Saoud
)
)
)

**AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME**

Assistant Commissioner for Patents
Washington, D.C. 20231

RECEIVED

AUG 4 1998

CUSTOMER
SERVICE CENTER

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111;
2. Declaration Under 37 C.F.R. § 1.132 of Dr. Kari Alitalo;
3. Declaration of Biological Culture Deposit in Compliance with Budapest Treaty Requirements;
4. Check in the amount of \$55.00 in payment of fee for extension of time; and
5. Check in the amount of \$159.00 in payment of fee for extra claims.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on July 23, 1998, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass.

1. **Small Entity Status**

☒ Small entity status has been established and is still effective.

2. **Extension of Time**

☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month		\$110.00	X	\$55.00
Two Months		\$400.00		\$200.00
Three Months		\$950.00		\$475.00
Four Months		\$1,510.00		\$755.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$55.00

☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$_____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$0

Extension Fee Due With This Request \$55.00

3. Fee for Claims

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	40	MINUS	33	7	X11 =	\$77	X22 =	\$
INDEP.	7	MINUS	5	2	X41 =	\$82	X82 =	\$
<input type="checkbox"/>	First Presentation of Multiple Dependent Claim				+ 135 =		+ 270 =	\$
TOTAL ADDITIONAL FEE						\$159	OR	\$

4. Method of Payment of Fees

- ☒ Attached are checks in the amount of \$55.00 and \$159.00.
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

5. Deposit Account and Refund Authorization


The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:


David A. Gass
Reg. No: 38,153

July 23, 1998

Jul. 23. 1998 5:15PM MARSHALL, OTOOLE

No. 8729 P. 1/4
From: 0819

MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BORUN

ATTORNEYS AT LAW
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606-6402
(312) 474-6300
FAX: (312) 474-0448

July 23, 1998

FACSIMILE TRANSMITTAL SHEET

TO: Examiner Saoud - Group Art Unit: 1646
c/o U.S. Patent and Trademark Office
(703) 308-0294
U.S. Serial No. 08/585,895

CLIENT NO: 28967
MATTER NO: 33072
COUNTRY CODE: US

FROM: David A. Gass
Marshall, O'Toole

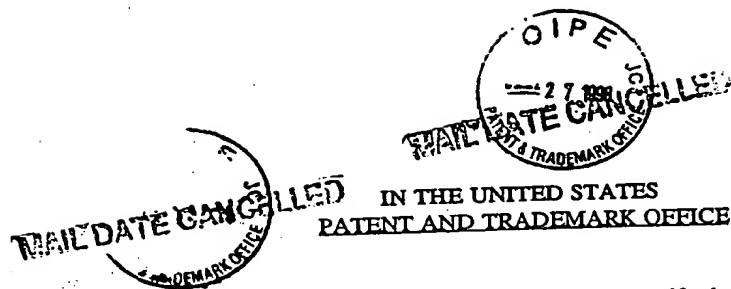
PAGES (INCLUDING THIS PAGE): 4

PLEASE CONFIRM RECEIPT: No.

MESSAGE: The attached declaration, which is a duplicate of a declaration filed November 26, 1997, was referenced in amendment papers filed today by first class mail in the above matter, but may have been inadvertently omitted.

Please contact Lisa Richard at (312) 474-6819 if you do not receive all of the pages in good condition.

The material of this transmission contains confidential information intended only for the addressee. If you are not the addressee, any disclosure or use of this information by you is strictly prohibited. If you have received this facsimile in error, please notify us by telephone immediately.



PATENT
28967/33072



In re Application of:

Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996

Title: RECEPTOR LIGAND

Art Unit: 1646

Examiner: Saoud

I hereby certify that this paper is
deposited with the United States Postal
Service as first class mail, postage
prepaid, in an envelope addressed to:
Assistant Commissioner for Patents
Washington, D.C. 20231, on this date:

Dated: July 23, 1998


David A. Gass

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. KARI ALITALO

I, Kari Alitalo, do hereby declare and state as follows:

1. I am a co-inventor of the above-identified U.S. Patent Application (hereinafter "the patent application"). I am familiar with the Office action from the U.S. Patent and Trademark Office dated March 24, 1998, in the patent application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the issues and rejections raised in the Office action.

2. I understand that sequences identified as SEQ ID NOs: 44 and 45 were added to the patent application by an amendment dated November 26, 1997, and entered by the Patent Office on December 1, 1997. Copies of those two sequences are appended hereto. I understand that, at the time of the amendment, SEQ ID NOs: 44 and 45 were identified as a nucleotide sequence and a deduced amino acid sequence of a cDNA that was deposited with the American Type Culture Collection (ATCC) as plasmid pFLT4-L and that is cross-referenced in the patent application at pages 28-29. I understand that the Patent Office has objected to the amendment to introduce these two sequences into the patent application on the

basis that such an amendment "introduces new matter into the disclosure." The Patent Office's basis for this allegation was as follows:

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97231 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO: 44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO: 44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "the approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO: 44."

(Office action dated March 24, 1998, at paragraph 10.)

3. I confirm that our laboratory sequenced the insert of the same plasmid that was designated pFLT4-L and that was deposited with the ATCC as ATCC Deposit No. 97231 and that is referred to at pages 28-29 of the patent application. The nucleotide sequence of the insert of this plasmid (ATCC Deposit No. 97231) includes the 1997 nucleotides of sequence set forth in SEQ ID NO: 44 as appended hereto and added to the patent application in the amendment dated November 26, 1997. The 419 residue amino acid sequence set forth in SEQ ID NO: 45 (as appended hereto and added to the patent application) is deduced from the sequence set forth in SEQ ID NO: 44.

4. The insert of plasmid pFLT4-L (ATCC Deposit No. 97231) contains additional (non-coding) sequence adjacent to the 1997 nucleotides of sequence set forth in SEQ ID NO: 44. The apparent size discrepancy between the approximately 2.1 kb size of the insert (as estimated by agarose gel electrophoresis analysis) and the 1997 nucleotides of sequence as set forth in SEQ ID NO: 44 is explained by the existence of this additional non-coding sequence in the plasmid insert.

Certification

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and

the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

July 17, 1998
Date

Kari Alitalo
Kari Alitalo

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1997 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CCCGCCCCGC CTCTCCAAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCTCGGCC	60
CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCCGGTTT CCTGTGAGGC	120
TTTTACTGA CACCCGCCGC TTTCCTCCGG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG	180
GGAACGCGGA GCCCCGGACC CGCTCCCGCC GCCTCCGGCT CGCCAGGGG GGGTCGCCGG	240
GAGGAGCCCG GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGGC GCCCGCGCCC	300
CCACCCCTGC CCCC GCCAGC GGACCGGTCC CCCACCCCGG GTCCTTCCAC C ATG CAC	357
Met His	
1	
TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG	405
Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu	
5 10 15	
CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC TTC GAG TCC	453
Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe Glu Ser	
20 25 30	
GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT	501
Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala	
35 40 45 50	
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA	549
Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val	
55 60 65	
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG	597
Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys	
70 75 80	
TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC	645
Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn	
85 90 95	

CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr 100 105 110	693
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln 115 120 125 130	741
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val 135 140 145	789
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys 150 155 160	837
GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr 165 170 175	885
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln 180 185 190	933
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg 195 200 205 210	981
TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg 215 220 225	1029
CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr 230 235 240	1077
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala 245 250 255	1125
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp 260 265 270	1173
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr 275 280 285 290	1221
TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro 295 300 305	1269
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys 310 315 320	1317

CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr 325 330 335	1365
TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn 340 345 350	1413
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu 355 360 365 370	1461
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg 375 380 385	1509
CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser 390 395 400	1557
GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met 405 410 415	1605
AGC TAAGATTGTA CTGTTTTCCA GTTCATCGAT TTTCTATTAT GGAAAACTGT Ser	1658
GTGCCACAG TAGAACTGTC TGTGAACAGA GAGACCCTTG TGGGTCCATG CTAACAAAGA	1718
CAAAAGTCTG TCTTTCTGA ACCATGTGGA TAACITTACA GAAATGGACT GGAGCTCATC	1778
TGCAAAAGGC CTCTGTAAA GACTGGTTTT CTGCCAATGA CCAACAGCC AAGATTTTCC	1838
TCTGTGATT TCTTTAAAG AATGACTATA TAATTTATTT CCACTAAAAA TATTGTTTCT	1898
GCATTCAITT TTATAGCAAC AACAATTGGT AAAACTCACT GTGATCAATA TTTTATATC	1958
ATGCAAAATA TGTTTAAAT AAAATGAAAA TTGTATTAT	1997

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 419 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala	
1 5 10 15	
Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe	
20 25 30	

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
 35 40 45
 Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
 50 55 60
 Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
 65 70 75 80
 Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
 85 90 95
 Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
 100 105 110
 His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
 115 120 125
 Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
 130 135 140
 Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
 145 150 155 160
 Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
 165 170 175
 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
 180 185 190
 Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
 195 200 205
 Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
 210 215 220
 Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
 225 230 235 240
 Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
 245 250 255
 Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
 260 265 270
 Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
 275 280 285
 Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
 290 295 300
 Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
 305 310 315 320
 Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
 325 330 335

Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
340 345 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
355 360 365

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
370 375 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
385 390 395 400

Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
405 410 415

Gln Met Ser



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/585,895	01/12/98	ALITALIA	28113/33072

Mail 1/10/98
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6000 GEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO, IL 60606-6402

EXAMINER

ART. UNIT. PAPER NUMBER

27
10/08/98

DATE MAILED:

Please find below a communication from the EXAMINER in charge of this application.

Commissioner of Patents

Applicant's response filed 27 July 1998 has been received. However, a reference relevant to the examination of this application may soon become available. *Ex parte* prosecution is SUSPENDED INDEFINITELY from the date of this letter. Applicant should feel free to make an inquiry as to the status of the application if needed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Saoud, Ph.D., whose telephone number is (703) 305-7519. The examiner can normally be reached on Monday to Friday from 8AM to 3PM.

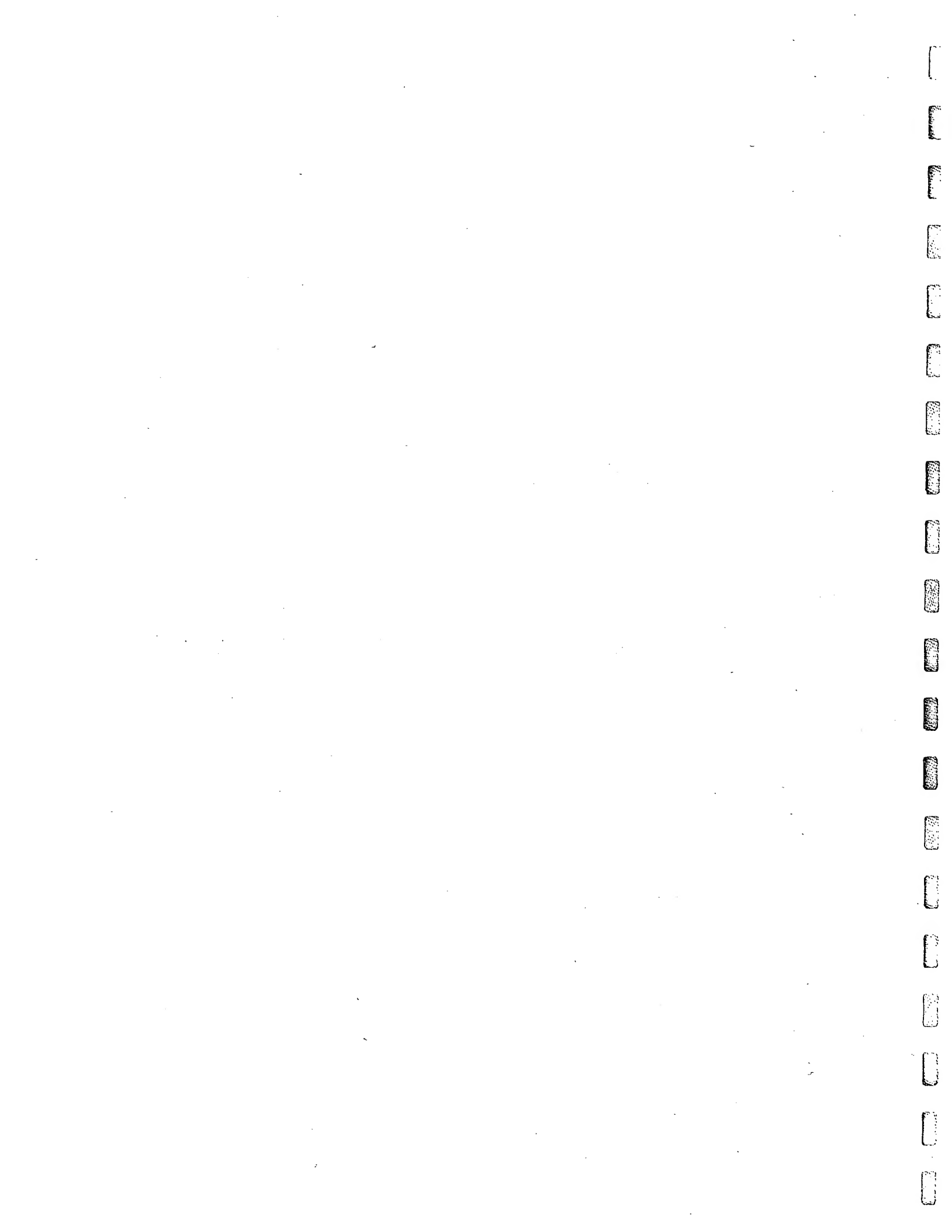
If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lila Feisee, can be reached on (703) 308-2731. The fax phone number for this Group is (703) 308-0294.

Official papers filed by fax should be directed to (703) 308-4227. Faxed draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Christine Saoud, Ph.D.
October 6, 1998

JOHN ULM
PRIMARY EXAMINER
GROUP 1800





Class 1646

PATENT
Attorney Docket No. 28967733072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

JUL 29 1999
TECH CENTER 1100 22

In the Application of: Kari Alitalo

and Vladimir Joukov

Serial No.: 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Group Art Unit: 1646

Examiner: Saoud, C.

) I hereby certify that this paper and
) the documents referred to as
) enclosed herewith are being
) deposited with the United States
) Postal Service as First Class Mail,
) postage prepaid, in an envelope
) addressed to: Assistant
) Commissioner for Patents,
) Washington, DC 20231, on this
) date:

) July 26, 1999

) Jill E. Uhl
) Jill E. Uhl

) Reg. No.: 43,213

) Attorney for Applicants

**SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith are a Form PTO-1449 listing several documents,
together with a copy of each listed document. The Applicants respectfully request that
these documents be made of record and considered in the above-identified application.

Documents A3-A6 are U.S. priority documents of published PCT
applications that are now publically available from WIPO.

Documents B9-B11, C117, C119, and C154-C157 were identified by the
European Patent Office in an International Search Report for a related PCT application. A

copy of the search report is also attached hereto.

Documents C120-C153 pertain to sequences, such as EST's, that have been posted in the Genbank Database, where the sequences should be available in computer readable form.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

Please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

July 26, 1999

By:

Jill E. Uhl
Jill E. Uhl
Registration No.: 43,213
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



PATENT

Attorney Docket No. 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Kari Alitalo

and Vladimir Joukov

Serial No.: 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Group Art Unit: 1646

Examiner: Saoud, C.

) I hereby certify that this paper and
) the documents referred to as
) enclosed herewith are being
) deposited with the United States
) Postal Service as First Class Mail,
) postage prepaid, in an envelope
) addressed to: Assistant
) Commissioner for Patents,
) Washington, DC 20231, on this
) date:

) July 26, 1999

) Jill E. Uhl
) Jill E. Uhl

) Reg. No.: 43,213

) Attorney for Applicants

**SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith are a Form PTO-1449 listing several documents,
together with a copy of each listed document. The Applicants respectfully request that
these documents be made of record and considered in the above-identified application.

Documents A3-A6 are U.S. priority documents of published PCT
applications that are now publically available from WIPO.

Documents B9-B11, C117, C119, and C154-C157 were identified by the
European Patent Office in an International Search Report for a related PCT application. A

copy of the search report is also attached hereto.

Documents C120-C153 pertain to sequences, such as EST's, that have been posted in the Genbank Database, where the sequences should be available in computer readable form.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

Please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

July 26, 1999

By:

Jill E. Uhl
Jill E. Uhl
Registration No.: 43,213
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

FILE COPY

#28

SHEET 1 of 5

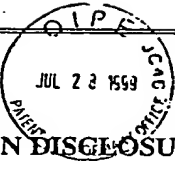
Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	App. Docket No. 28967/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo, K. et al.	
		Filing Date January 12, 1996	Group 1646

U.S. PATENT DOCUMENTS							
*Examiner Initials		Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate
ck	A3	08/207,550	none	Jing-Shan Hu and Liang Cao			03/08/94
ck	A4	08/465,968	none	Crain Rosen, Jing-Shan Hu and Liang Cao			06/06/95
ck	A5	60/003,491	none	James Lee and William Wood			09/08/95
ck	A6	08/554,374	none	Lyman, S.			11/08/95

FOREIGN PATENT DOCUMENTS								
*Examiner Initials		Document Number	Publication Date	Country	Class	Subclass	Translation	
							Yes	No
ck	B8	0 506 477 A1	03/27/92	EP				
ck	B9	97/05250 A	02/13/97	WO				
ck	B10	97/09427 A	03/13/97	WO				
ck	B11	97/17442 A	05/15/97	WO				

EXAMINER <i>C. Saoud</i>	DATE CONSIDERED <i>3/28/00</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

SHEET 2 of

Form PTO-1449 (Modified)		U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)			Applicant Alitalo, K. <i>et al.</i>	
			Filing Date January 12, 1996	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
u	C117	Achen, M.G. <i>et al.</i> , "Vascular Endothelial Growth Factor D (VEGF-D) is a Ligand for the Tyrosine Kinases VEGF Receptor 2 (Flk1) and VEGF Receptor 3 (Flt4)," <i>Proceedings of the National Academy of Science, USA</i> , 95:548-553 (January, 1998).
	C118	Adams, M.D. <i>et al.</i> , "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence," <i>Nature</i> , 377(6547 Supplement):3-174 (September, 1995).
	C119	Cohen, T. <i>et al.</i> , "VEGF ₁₂₁ , A Vascular Endothelial Growth Factor (VEGF) Isoform Lacking Heparin Binding Ability, Requires Cell-Surface Heparan Sulfates for Efficient Binding to the VEGF Receptors of Human Melanoma Cells," <i>Journal of Biological Chemistry</i> , 270(19):11322-11326 (May 12, 1995).
	C120	Genbank AA151613, "z127h03.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 3'," Hillier, L. <i>et al.</i> , Dated 14-May-1997
	C121	Genbank AA425486, "zw46b06.r1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 5' similar to SW:VEGF_MOUSE Q00731 VASCULAR ENDOTHELIAL GROWTH FACTOR PRECURSOR," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997
	C122	Genbank N31713, "yy15b12.s1 Homo sapiens cDNA clone 271295 3'," Deposited by Hillier, L. <i>et al.</i> Dated 10-Jan-1996
	C123	Genbank N31720, "yy15d12.s1 Homo sapiens cDNA clone 271319 3'," Deposited by Hillier, L. <i>et al.</i> Dated 10-Jan-1996
	C124	Genbank AA406492, "zv12g06.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 75366 5'," Deposited by Hillier, L. <i>et al.</i> Dated 17-May-1997
	C125	Genbank N50972, "yy94b08.s1 Homo sapiens cDNA clone 281175 3'," Deposited by Hillier, L. <i>et al.</i> Dated 14-Feb-1996
u	C126	Genbank AA421713, "zu24b03.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 738893 3'," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997

EXAMINER	C. Saoud	DATE CONSIDERED	3/28/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.			

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date January 12, 1996	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
✓	C127	Genbank N94399, "zb76f04.s1 Soares senescent fibroblasts NbHSF Homo sapiens cDNA clone 309535 3'," Deposited by Hillier, L. <i>et al.</i> Dated 20-Aug-1996
	C128	Genbank H05177, "y185b08.r1 Homo sapiens cDNA clone 44993 5'," Deposited by Hillier, L. <i>et al.</i> Dated 21-Jun-1995
	C129	Genbank AA479987, "zv18h12.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 3'," Deposited by Hillier, L. <i>et al.</i> Dated 08-Aug-1997
	C130	Genbank H05134, " y185b08.s1 Homo sapiens cDNA clone 44993 3'," Deposited by Hillier, L. <i>et al.</i> Dated 21-Jun-1995
	C131	Genbank, AA298182 "EST113866 Bone VII Homo sapiens cDNA 5' end," Deposited by Adams, M.D. <i>et al.</i> Dated 18-Apr-1997
	C132	Genbank AA298283, "EST113896 Bone VII Homo sapiens cDNA 5' end similar to similar to vascular endothelial growth factor," Deposited by Adams, M.D. <i>et al.</i> Dated 18-Apr-1997
	C133	Genbank T81481, "yd29f07.s1 Homo sapiens cDNA clone 109669 3'," Deposited by Hillier, L. <i>et al.</i> Dated 15-Mar-1995
	C134	Genbank AA425303, "zw46b06.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 3', mRNA sequence," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997
	C135	Genbank Z40230, "H. sapiens partial cDNA sequence; clone c-1wf11," Deposited by Genexpress. Dated 21-Sep-1995
	C136	Genbank Z44272, "H. sapiens partial cDNA sequence; clone c-1wf11," Deposited by Genexpress. Dated 21-Sep-1995
	C137	Genbank AA478766, " zv18h12.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 5'," Deposited by Hillier, L. <i>et al.</i> Dated 08-Aug-1997
✓	C138	Genbank H96876, "yw04b12.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 3'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Nov-1996

EXAMINER C. Saoud	DATE CONSIDERED 3/28/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)

U.S. Department of Commerce
Patent and Trademark OfficeAny. Docket No.
28967/33072Serial No.
08/585,895

INFORMATION DISCLOSURE STATEMENT

(Use several sheets if necessary)

Applicant
Alitalo, K. *et al.*Filing Date
January 12,
1996Group
1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

C139	Genbank H96533, "yw04b12.r1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Nov-1996
C140	Genbank T81690, "yd29f07.r1 Homo sapiens cDNA clone 109669 5' similar to SP:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3," Deposited by Hillier, L. <i>et al.</i> Dated 15-Mar-1995
C141	Genbank T84377, "yd37h08.r1 Homo sapiens cDNA clone 110463 5' similar to SP:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3," Deposited by Hillier, L. <i>et al.</i> Dated 16-Mar-1995
C142	Genbank N42368, "yy15b11.r1 Homo sapiens cDNA clone 271293 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Jan-1996
C143	Genbank N42374, "yy15d11.r1 Homo sapiens cDNA clone 271317 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Jan-1996
C144	Genbank H81868, "yv83d09.s1 Homo sapiens cDNA clone 249329 3'," Deposited by Hillier, L. <i>et al.</i> Dated 09-Nov-1995
C145	Genbank H81867, "yv83d09.r1 Homo sapiens cDNA clone 249329 5'," Deposited by Hillier, L. <i>et al.</i> Dated 09-Nov-1995
C146	Genbank AA149461, "z127h03.r1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 5' similar to SW:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3 PRECURSOR," Deposited by Hillier, L. <i>et al.</i> Dated 14-May-1997
C147	Genbank R77495, "yi79e04.s1 Homo sapiens cDNA clone 145470 3'," Deposited by Hillier, L. <i>et al.</i> Dated 07-Jun-1995
C148	Genbank H07899, "y186g06.s1 Homo sapiens cDNA clone 45138 3'," Deposited by Hillier, L. <i>et al.</i> Dated 23-Jun-1995
C149	Genbank T89295, "yd37h08.s1 Homo sapiens cDNA clone 110463 3'," Deposited by Hillier, L. <i>et al.</i> Dated 20-Mar-1995
C150	Genbank C21512, "HUMGS0010510, Human Gene Signature, 3'-directed cDNA sequence," Deposited by Okubo, K. Dated 01-Oct-1996

EXAMINER

C. Saoud

DATE CONSIDERED

3/28/96

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

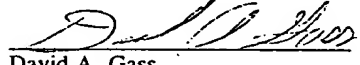
Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date January 12, 1996	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
✓	C151	Genbank N82975, "TgESTzy53h10.r1 TgRH Tachyzoite cDNA Toxoplasma gondii cDNA clone tgzy53h10.r1 5'," Deposited by Hehl, A. <i>et al.</i> Dated 10-Sep-1997
	C152	Genbank AA285997, "vb88h06.r1 Soares mouse 3NbMS Mus musculus cDNA clone 764123 5'," Deposited by Marra, M. <i>et al.</i> Dated 09-Apr-1997
	C153	Genbank AAS49856, "0929m3 gmbPfHB3.1, G. Roman Reddy Plasmodium falciparum genomic clone 0929m," Deposited by Dame, J.B. <i>et al.</i> Dated 11-Aug-1997
	C154	Jeltsch, M. <i>et al.</i> , "Hyperplasia of Lymphatic Vessels in VEGF-C Transgenic Mice," <i>Science</i> , 276:1423-1425 (May, 1997).
	C155	Joukov, V. <i>et al.</i> , "Proteolytic Processing Regulates Receptor Specificity and Activity of VEGF-C," <i>EMBO Journal</i> , 16(13):3898-3911 (June, 1997).
	C156	Joukov, V. <i>et al.</i> , "A Recombinant Mutant Vascular Endothelial Growth Factor-C that has Lost Vascular Endothelial Growth Factor Receptor-2 Binding, Activation, and Vascular Permeability Activities," <i>Journal of Biological Chemistry</i> , 273(12):6599-6602 (March 20, 1998).
✓	C157	Lee, J. <i>et al.</i> , "Vascular Endothelial Growth Factor Related Protein (vrp): A Ligand and Specific Activator of the Tyrosine Kinase Receptor Flt4," EMBL Sequence Data Library, XP002066361, accession no. U4142. Dated 10-Jan-1996

EXAMINER C Saoud	DATE CONSIDERED 3/28/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

7/18/99
PATENT
Attorney Docket No: 28967/33072
8:34

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Kari Alitalo)	I hereby certify that this paper and the
)	documents referred to as enclosed
and Vladimir Joukov)	herewith are being deposited with the
)	United States Postal Service as First
Serial No.: 08/585,895)	Class Mail, postage prepaid, in an
)	envelope addressed to: Assistant
Filed: January 12, 1996)	Commissioner for Patents,
)	Washington, DC 20231, on this date:
For: RECEPTOR LIGAND)	
)	October 26, 1999
Group Art Unit: 1646)	
)	
Examiner: Saoud, C.)	
)	David A. Gass
)	Reg. No.: 38,153
)	Attorney for Applicants

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The applicants request that the documents listed on the attached Form PTO-1449 be made of official record and considered by the Examiner in the above-identified application.

Pursuant to 37 C.F.R. §1.98(d), copies of all listed documents are not enclosed because they were cited in a prior application (U.S. Serial No. 08/510,133, filed August 1, 1995) that is presently relied upon herein for an earlier filing date. However, copies of these documents will be resubmitted at the Examiner's request.

This Supplemental Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.


Pursuant to 37 C.F.R. §1.97(e)(2), the listed documents were not known to the applicants or to any individual designated in §1.56 (c) as issued U.S. patents more than three months prior to the filing of this Supplemental Information Disclosure Statement, because U.S. Patent No. 5,932,540 (document A7) issued on August 3, 1999 and U.S. Patent No. 5,935,820 (document A8) issued on August 10, 1999. Consequently, this Supplemental Information Disclosure Statement should be considered by the Patent Office without payment of a fee. However, please charge any necessary fees due in connection with this Supplemental Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300


October 26, 1999

By:


David A. Gass
Reg. No.: 38,153

PATENT
Attorney Docket No. 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Kari Alitalo)	I hereby certify that this paper and the
)	documents referred to as enclosed
and Vladimir Joukov)	herewith are being deposited with the
)	United States Postal Service as First
Serial No.: 08/585,895)	Class Mail, postage prepaid, in an
)	envelope addressed to: Assistant
Filed: January 12, 1996)	Commissioner for Patents,
)	Washington, DC 20231, on this date:
For: RECEPTOR LIGAND)	
)	October 26, 1999
Group Art Unit: 1646)	
)	
Examiner: Saoud, C.)	
)	David A. Gass
)	Reg. No.: 38,153
)	Attorney for Applicants

**SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The applicants request that the documents listed on the attached Form PTO-1449 be made of official record and considered by the Examiner in the above-identified application.

Pursuant to 37 C.F.R. §1.98(d), copies of all listed documents are not enclosed because they were cited in a prior application (U.S. Serial No. 08/510,133, filed August 1, 1995) that is presently relied upon herein for an earlier filing date. However, copies of these documents will be resubmitted at the Examiner's request.

This Supplemental Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

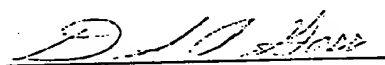
Pursuant to 37 C.F.R. §1.97(e)(2), the listed documents were not known to the applicants or to any individual designated in §1.56 (c) as issued U.S. patents more than three months prior to the filing of this Supplemental Information Disclosure Statement, because U.S. Patent No. 5,932,540 (document A7) issued on August 3, 1999 and U.S. Patent No. 5,935,820 (document A8) issued on August 10, 1999. Consequently, this Supplemental Information Disclosure Statement should be considered by the Patent Office without payment of a fee. However, please charge any necessary fees due in connection with this Supplemental Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

October 26, 1999

By:


David A. Gass
Reg. No.: 38,153

FILE COPY

#2
SHEET 1 of 1

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/33072	Serial No. 08/585,895
INFORMATION DISCLOSURE STATEMENT 3 (Use several sheets if necessary)		Applicant Alitalo, K. et al.	
		Filing Date January 12, 1996	Group 11646

U.S. PATENT DOCUMENTS							
*Examiner Initials		Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate
CK	A7	5,932,540	08/03/99	Jing-Shan Hu <i>et al.</i>	514	2	
CK	A8	5,935,820	08/10/99	Jing-Shan Hu <i>et al.</i>	435	69.4	

FOREIGN PATENT DOCUMENTS								
*Examiner Initials		Document Number	Publication Date	Country	Class	Subclass	Translation	
							Yes	No

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		

EXAMINER C. Saoud	DATE CONSIDERED 3/28/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/585,895	01/12/96	ALITALO	K 28113/33072

HM22/0404
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

EXAMINER

SAOUD, C

ART UNIT	PAPER NUMBER
----------	--------------

1646

3c

DATE MAILED: 04/04/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.
08/585,895

Applicant(s)

Alitalo et al.

Examiner

Christine Saoud

Group Art Unit
1646

- ☐ Responsive to communication(s) filed on _____
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.
- A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

- ☒ Claim(s) 1, 3-5, 7, 11, and 18-44 is/are pending in the application.
- Of the above, claim(s) _____ is/are withdrawn from consideration.
- ☐ Claim(s) _____ is/are allowed.
- ☒ Claim(s) 1, 3-5, 7, 11, and 18-44 is/are rejected.
- ☐ Claim(s) _____ is/are objected to.
- ☐ Claims _____ are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been
- ☐ received.
- ☐ received in Application No. (Series Code/Serial Number) _____.
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
- *Certified copies not received: _____
- ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☐ Notice of References Cited, PTO-892
- ☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 28 and 29
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Notice of Informal Patent Application, PTO-152

— SEE OFFICE ACTION ON THE FOLLOWING PAGES —

Application/Control Number: 08/585,895

Page 2

Art Unit: 1646

DETAILED ACTION

Response to Amendment

1. Claims 1, 3-5, 7, 18, 19, 26-33, and 36-37 have been amended and claims 39-44 have been added as requested in the amendment of paper #26, filed 27 July 1998. Claims 1, 3-5, 7, 11, and 18-44 are pending in the instant application.
2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
3. Any objection or rejection of record which is not expressly repeated in this action has been overcome by Applicant's response and withdrawn.
4. Applicant's arguments filed 27 July 1998 have been fully considered, however, in light of the new grounds of rejection below, the arguments are not found to be relevant and therefore, have not been addressed.

Claim Rejections - 35 USC § 112

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to

Application/Control Number: 08/585,895

Page 3

Art Unit: 1646

make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 1, 37, and 42 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1 and 42 and dependent claim 37 are directed to subject matter of a polynucleotide that hybridizes to a DNA under specific conditions which are recited in the claims, wherein the polynucleotide encodes a protein which has particular structural and functional features. In making a determination of whether the application complies with the written description requirement of 35 U.S.C. 112, first paragraph, it is necessary to understand what Applicant has possession of and what Applicant is claiming. From the specification, it is clear that Applicant has possession of a nucleic acid molecule which encodes a protein which has the amino acid sequence of SEQ ID NO:33. This nucleic acid molecule has a nucleic acid sequence of SEQ ID NO:32 and is contained within plasmid pFLT4-L (ATCC deposit #97231). The subject matter which is claimed is described above. First, a determination of the level of predictability in the art must be made in that whether the level of skill in the art leads to a predictability of structure; and/or whether teachings in the application or prior art lead to a predictability of structure. The claims are directed to host cells which are transfected with a polynucleotide which encodes a polypeptide, wherein the polynucleotide hybridizes to a DNA of SEQ ID NO:32 under a specific set of hybridization conditions. First, the claims are not limited to polynucleotide molecules

Art Unit: 1646

encoding a protein with a specific amino acid sequence. The claims only require the nucleic acid molecule to encode a polypeptide which belongs to the VEGF/PDGF family (implied by the recitation of the 8 cysteine domain) and which is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase. The specification only describes a single polypeptide from a human and fails to teach or describe any other polypeptide which has the structural and functional characteristics recited in the claims. The breadth of the claims is such that the claims encompass polynucleotides from other species and polynucleotides which encode variant polypeptides so long as receptor binding activity is maintained. There is a lack of guidance or teaching regarding structure and function because there is only a single example provided in the specification and because there is no guidance found in the prior art. The claims include polynucleotides which share some sequence similarity to the disclosed polynucleotide which encodes the polypeptide of SEQ ID NO:33, however, this sequence similarity is not sufficient to provide the function of encoding a polypeptide which binds to the Flt4 receptor tyrosine kinase.

Next in making a determination of whether the application complies with the written description requirement of 35 U.S.C. 112, first paragraph, each claimed species and genus must be evaluated to determine whether there is sufficient written description to inform a skilled artisan that applicant was in possession of the claimed invention at the time the application was filed. With this regard, the instant application fails to provide a written description of the species or the genus which are encompassed by the instant claims except for the nucleic acid of SEQ ID NO:32. The specification does not provide a complete structure of those polynucleotides which encode a

Application/Control Number: 08/585,895

Page 5

Art Unit: 1646

polypeptide as described in the claims and hybridize to the recited sequence under the recited stringency conditions of the claims. The claims also fail to recite other relevant identifying characteristics (physical and/or chemical and/or functional characteristics coupled with a known or disclosed correlation between function and structure) sufficient to describe the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention. The specification fails to provide a representative number of species for the claimed genus (those polynucleotides which hybridize to SEQ ID NO:32 under the recited stringency conditions) because the claims are directed to those polynucleotides which encode a polypeptide having a conserved cysteine domain and which binds to the human Flt4 receptor tyrosine kinase, which encompasses different species and variants and the specification teaches one embodiment. Therefore, the claims are directed subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim Rejections - 35 USC § 102

7. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless —

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who

Art Unit: 1646

has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

8. Claims 1, 3-5, 7, 11, and 18-44 are rejected under 35 U.S.C. 102(e) as being anticipated by Hu et al. (U.S. Pat. No. 5,935,820).

Hu et al. disclose a polynucleotide, SEQ ID NO:1, which encodes a polypeptide, SEQ ID NO:2, which includes a domain defined by 8 cysteine residues of the VEGF family, and which is capable of binding to human Flt4 receptor tyrosine kinase. The instant claims indicate that the polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein, however, this limitation only further defines the processed protein and places no material limitations on the polynucleotide. Claim 11 further defines the polypeptide as comprising amino acids 1 to 120 of SEQ ID NO:33, however, this limitation places no material limitations on the polynucleotide. Claim 18 is directed to a polynucleotide which lacks a portion of the nucleic acid sequence which encodes the cysteine motifs of a Balbiani ring 3 protein, but still encodes a polypeptide that is capable of binding to human Flt4 receptor tyrosine kinase. This limitation appears to be inherently met by the embodiment of claim 1 of '820 in that the mature protein lacks this portion of the polypeptide, therefore, a "polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2" anticipates this claim.

Allowable Subject Matter

9. It is noted that some of the claims appear to be directed to polynucleotides encoding a polypeptide comprising amino acids 1-120 of SEQ ID NO:33. The prior art does not disclose or

Application/Control Number: 08/585,895

Page 7

Art Unit: 1646

teach a polypeptide consisting of amino acids 1-120 of SEQ ID NO:33. Specific claims to the embodiment of polynucleotide encoding a polypeptide consisting of amino acids 1-120 of SEQ ID NO:33 appear to be free of the prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Saoud, Ph.D., whose telephone number is (703) 305-7519. The examiner can normally be reached on Monday to Friday from 8AM to 3PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Kunz, can be reached on (703) 308-4623. The fax phone number for this Group is (703) 308-0294.

Official papers filed by fax should be directed to (703) 308-4227. Faxed draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

April 3, 2000

**CHRISTINE SAOUD
PATENT EXAMINER**

Christine Saoud

PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)

Serial No: 08/585,895)

Filed: January 12, 1996)

Title: Receptor Ligand)

Group Art Unit: 1646)

Examiner: Christine Saoud)

ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned attorney of record in the above-identified application
hereby appoints as associate attorney(s):

Frank S. DiGiglio (Reg. No. 31,346)
Scully, Scott, Murphy & Presser
400 Garden City Plaza
Garden City, New York 11530
(516) 742-4343

to prosecute this application, to make alterations or amendments therein, and to
transact any and all business in the Patent and Trademark Office connected
therewith.

MARSHALL, OTCOLE, GERSTEIN,
MURRAY & BORUN



David A. Gass
Registration No. 38,153

June 22, 2000



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/585,895	01/12/96	ALITALIA	28113/30072

HM22/0629

FRANK S. DIGIELLO
SCULLY SCOTT MURPHY & PRESSER
400 GARDEN CITY PLAZA
GARDEN CITY NY 11530

EXAMINER

SADUD, C

ART UNIT

1647

PAPER NUMBER

32

DATE MAILED: 06/29/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
--------------------	-------------	-----------------------	---------------------

08/585,895

EXAMINER

ART UNIT	PAPER NUMBER
----------	--------------

31

DATE MAILED:

INTERVIEW SUMMARY

All participants (applicant, applicant's representative, PTO personnel):

(1) Christine Saoud (3) DAVID GASS
(2) Gary Kunz (4) WILLIAM MERHEL
Date of Interview June 22, 2000 (5) FRANK J. DiGiglio

Type: ☐ Telephonic ☒ Personal (copy is given to ☐ applicant ☒ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No If yes, brief description: _____

Agreement ☐ was reached. ☒ was not reached.

Claim(s) discussed: 1, 3, 33, 18

Identification of prior art discussed: Hu et al. - of record in last office action.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Discussed clms w/ hybridization language as it relates to written description and enablement for prod. which binds Flt 4. Discussed host cell clms and product of mature (truncated) VEGF-C. Host cells which produce mature VEGF-C distinguish over the prior art of record.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign this form unless it is an attachment to another form.

FORM PTOL-413 (REV. 1-99)

Christine Saoud



FAU. 1646 \$
RECEIVED

AUG 15 2000

TECH CENTER 1600/2500

PATENT

#33
177

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):)	Title: RECEPTOR LIGAND
Alitalo et al.)	
Serial No: 08/585,895)	Group Art Unit: 1646
Filed: January 12, 1996)	Examiner: Christine Saoud

AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111;
2. Declaration Pursuant to 37 C.F.R. § 1.132 of Kari Alitalo (unsigned); and
3. Check in the amount of \$91.00 in payment of fee for extension of time (\$55.00) and fee for extra claims (\$36.00).

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 4, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

David A. Gass

08/11/2000 JADD01 00000011 08585895

01 FC:215

55.00 DP

RECEIVED
 11-11-11
 RECEIVED

1. **Small Entity Status**

Verified statement(s) claiming small entity status is(are) attached.

☒ Small entity status has been established and is still effective.

Has not been established.

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For	RATE	FEE	RATE	FEE
TOTAL	44	MINUS 40 = 4	X 9 =	\$36.00	X 18 =	\$
INDEP.	7	MINUS 7 = 0	X 39 =	\$	X 78 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 130 =	\$	+ 260 =	\$
Filing Fee:				\$36.00	OR	\$

2. **Extension of Time**

☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY	FEE FOR SMALL ENTITY
One Month	\$110.00	X \$55.00
Two Months	\$380.00	\$190.00
Three Months	\$870.00	\$435.00
Four Months	\$1,360.00	\$680.00
Five Months	\$1,850.00	\$925.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: **\$55.00**

☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$ _____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$

RECEIVED

AUG 15 2000

TECH CENTER 1600/2

Extension Fee Due With This Request: \$55.00

3. Method of Payment of Fees

- ☒ Attached is a check in the amount of \$91.00
- ☐ Charge Deposit Account No. 13-2855
in the amount of: \$ _____
A copy of this Petition is enclosed.

4. Deposit Account and Refund Authorization

- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 to Deposit Account No. 13-2855. A copy of this Petition is enclosed.
- ☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 

David A. Gass
Reg. No: 38,153

August 4, 2000



RECEIVED
AUG 15 2000
TECH CENTER 16000570
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):)	Title: RECEPTOR LIGAND
Alitalo et al.)	
Serial No: 08/585,895)	Group Art Unit: 1646
Filed: January 12, 1996)	Examiner: Christine Saoud

AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111;
2. Declaration Pursuant to 37 C.F.R. § 1.132 of Kari Alitalo (unsigned); and
3. Check in the amount of \$91.00 in payment of fee for extension of time (\$55.00) and fee for extra claims (\$36.00).

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 4, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

Verified statement(s) claiming small entity status is(are) attached.

- ☒ Small entity status has been established and is still effective.
 Has not been established.

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For	RATE	FEE	RATE	FEE
TOTAL	44	MINUS 40 = 4	X 9 =	\$36.00	X 18 =	\$
INDEP.	7	MINUS 7 = 0	X 39 =	\$	X 78 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 130 =	\$	+ 260 =	\$
Filing Fee:				\$36.00	OR	\$

2. **Extension of Time**

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month		\$110.00	X	\$55.00
Two Months		\$380.00		\$190.00
Three Months		\$870.00		\$435.00
Four Months		\$1,360.00		\$680.00
Five Months		\$1,850.00		\$925.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$55.00

- ☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$ _____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$

Extension Fee Due With This Request: \$55.00

3. Method of Payment of Fees

- ☒ Attached is a check in the amount of \$91.00
- ☐ Charge Deposit Account No. 13-2855
in the amount of: \$ _____
A copy of this Petition is enclosed.

4. Deposit Account and Refund Authorization

- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 to Deposit Account No. 13-2855. A copy of this Petition is enclosed.
- ☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: _____
David A. Gass
Reg. No: 38,153

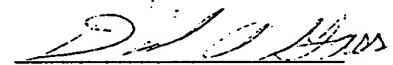
August 4, 2000



E/35
1/27
8/15/00

PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)	I hereby certify that this paper is being
Serial No: 08/585,895)	deposited with the United States Postal
Filed: January 12, 1996)	Service with sufficient postage as first class
Title: RECEPTOR LIGAND)	mail, postage prepaid, in an envelope
)	addressed to: Assistant Commissioner for
)	Patents, Washington, D.C., 20231 on this
)	date:
)	
Group Art Unit: 1646)	Date: August 4, 2000
Examiner: Christine Saoud)	
)	
)	David A. Gass
)	Registration No. 38,153
)	Attorney for Applicants
)	

AMENDMENT AND REPLY PURSUANT TO 37 C.F.R. §§ 1.111

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In an Office action mailed April 4, 2000, the Patent Office rejected claims 1, 3-5, 7, 11, and 18-44 variously under 35 USC §§ 102(e) and 112, first paragraph. The Applicants respectfully request reconsideration in light of the following amendments and remarks. This amendment is timely filed with a petition and fee for one month extension of time.

08/11/2000 JADD01 00000011 08585895

02 FC:203

36.00 CP

AMENDMENTS

In the claims:

Please cancel all pending claims and add new claims 45-79 as shown below:

~~45.~~ ¹ A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds to human Flt4 receptor tyrosine kinase (Flt4), said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

~~46.~~ ² A purified and isolated nucleic acid according to claim ~~45~~ ¹ wherein said polypeptide stimulates tyrosine phosphorylation of human Flt4.

~~47.~~ ³ A purified and isolated nucleic acid according to claim ~~46~~ ² wherein said polypeptide has an apparent molecular weight of about 23 kD as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

~~48.~~ ⁴ A purified and isolated nucleic acid according to claim ~~46~~ ² wherein said polypeptide comprises an amino-terminal amino acid sequence set forth in SEQ ID NO: 13.

~~49.~~ ⁵ A purified and isolated nucleic acid according to claim ~~48~~ ⁴ wherein said polypeptide comprises approximately 120 amino acids.

~~50.~~ ⁶ A purified and isolated nucleic acid according to claim ~~48~~ ⁴ wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence identical to amino acids 2 through 18 set forth in SEQ ID NO: 13.

~~51.~~ ⁷ A purified and isolated nucleic acid according to claim ~~46~~ ² wherein said polypeptide comprises amino acids 1 to 120 of SEQ ID NO: 33.

⁸
52. A purified and isolated nucleic acid according to claim ¹48 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

⁹
53. A nucleic acid according to claim ¹49 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes eight cysteines of SEQ ID NO: 33 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and excludes the carboxyl terminal portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

¹⁰
54. A nucleic acid according to claim ⁹53 wherein said continuous portion has amino acid 1 of SEQ ID NO: 33 as its amino terminus.

¹¹
55. A nucleic acid according to claim ¹54 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

¹²
56. A vector comprising a nucleic acid according to claim ¹55, wherein said vector lacks a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO:33 that has cysteine motifs of a Balbiani ring 3 protein.

¹³
¹²
57. A host cell transformed or transfected with a vector according to claim ¹56.

¹⁴
58. A method for producing a polypeptide that binds to the extracellular domain of human Flt4, comprising the steps of:
growing a host cell according to claim ¹³57 under conditions which permit expression by said host cell of a polypeptide that is encoded by said nucleic acid and that binds to the extracellular domain of human Flt4; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

¹⁵
~~55~~ 14 A method according to claim ~~55~~ wherein said host cell is a mammalian host cell that secretes said polypeptide and wherein said isolating step comprises isolating said polypeptide from said growth medium.

¹⁶
~~60~~ 16 A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds human Flt4 receptor tyrosine kinase (Flt4), said polypeptide having an amino acid sequence comprising a continuous portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

¹⁷
~~61~~ 17 A purified and isolated nucleic acid according to claim ~~60~~ ¹⁶ wherein said encoded polypeptide stimulates tyrosine phosphorylation of human Flt4.

¹⁸
~~62~~ 18 A purified and isolated nucleic acid according to claim ~~60~~ ¹⁶ wherein said nucleic acid lacks a nucleotide sequence that encodes the amino terminal portion of the amino acid sequence shown in SEQ ID NO: 33 that precedes residue 1.

¹⁹
~~63~~ 19 An expression construct comprising the nucleic acid according to claim ~~62~~ ¹⁸ operatively linked to an expression control sequence, said expression construct lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO:33 beyond residue 125.

²⁰
~~64~~ 20 A host cell transformed or transfected with the expression construct of claim ~~63~~ ¹⁹.

²¹
~~65~~ 21 A method for producing a polypeptide that binds to the extracellular domain of human Flt4 and stimulates tyrosine phosphorylation of Flt4, comprising the steps of:

growing a host cell according to claim ~~64~~ under conditions which permit expression in said host cell of a polypeptide encoded by said nucleic acid and isolating said polypeptide from the host cell or the growth medium of the host cell, wherein said polypeptide binds to the extracellular domain of human Flt4 and stimulates phosphorylation of Flt4.

²²
~~66~~. A host cell transformed or transfected with a polynucleotide, wherein said polynucleotide includes a strand containing a human nucleotide sequence that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

²¹
(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses a polypeptide encoded by said polynucleotide, wherein said polypeptide has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions and includes a domain encoded by the human nucleotide sequence that is defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B),

wherein said polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide binds to the extracellular domain of human Flt4 receptor tyrosine kinase.

²³
~~67~~. A host cell according to claim ²²~~66~~ that expresses a naturally occurring human Flt4 ligand polypeptide encoded by said polynucleotide.

²⁴
~~66~~. A host cell according to claim 66 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to sequence that encodes said polypeptide.

²⁵
~~67~~. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a contiguous portion of SEQ ID NO: 33 that is sufficient to bind to the extracellular domain of human Flt4 receptor tyrosine kinase (Flt4EC),

wherein said contiguous portion includes eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B),

wherein said polypeptide lacks any portion of SEQ ID NO: 33 that precedes position 1 and lacks any portion of SEQ ID NO: 33 that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide has a molecular weight of about 23 kD as assessed by SDS PAGE under reducing conditions and binds to Flt4EC.

²⁶
~~70~~. A host cell according to claim ²⁵~~68~~ wherein said nucleotide sequence comprises nucleotides 37 to 1086 of the sequence shown in SEQ ID NO: 32.

²⁷
~~71~~. A host cell according to claim ²⁵~~69~~ wherein said polynucleotide is a vector comprising an expression control sequence operatively linked to the nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO: 33.

²⁸
~~72~~. A eukaryotic host cell according to claim ²²~~66~~ or ²⁵~~69~~ that secretes said polypeptide.

²⁹
~~73~~. A host cell comprising the insert of plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses and secretes a polypeptide encoded by said insert,

wherein said secreted polypeptide has a molecular weight of about 23kD as assessed by SDS-PAGE under reducing conditions and binds to human Flt4 receptor tyrosine kinase and includes a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).

30
A. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that binds to the extracellular domain of human Flt4 receptor tyrosine kinase,

wherein said polynucleotide includes a strand containing a human nucleotide sequence that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

21
(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses and secretes a polypeptide encoded by said polynucleotide, and

wherein said expressed and secreted polypeptide binds the extracellular domain of human Flt4 receptor tyrosine kinase and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

31
B. A method for producing a polypeptide that binds the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:
growing a host cell according to any one of claims 25-30, or 32, under conditions which permit expression by said host cell of said polypeptide; and
isolating said polypeptide from the host cell or the growth medium of the host cell.

³² 76. A method for producing a polypeptide that binds to the extracellular domain (EC) of human Flt4 receptor tyrosine kinase (Flt4), comprising steps of:
growing a host cell comprising a polynucleotide that comprises a nucleotide sequence that encodes the amino acid sequence set forth in SEQ ID NO:33, under conditions in which the host cell expresses and secretes a polypeptide encoded by the polynucleotide; and
isolating a polypeptide that binds Flt4 EC from the growth medium of the host cell, said polypeptide having a molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions and having an amino acid sequence comprising a portion of SEQ ID NO:33 effective to bind Flt4 EC.

³³ 77. A method according to claim ³² 76 wherein said polynucleotide comprises an expression vector that comprises a nucleotide sequence that encodes the amino acid set forth in SEQ ID NO:33.

³⁴ 78. A method according to claim ³² 76 wherein said host cell comprises a PC-3 prostatic adenocarcinoma cell (ATCC CRL1435).

³⁵ 79. A method according to claim ³² 76 wherein said polynucleotide comprises the insert of plasma pFLT4-L, deposited as ATCC Accession No. 97231.--

REMARKS

I. Prosecution History.

The application as filed contained 16 claims. In an official communication dated November 25, 1996, claims 1-16 were subjected to a restriction requirement. In an Amendment and Election in Response to Restriction Requirement filed on January 24, 1997, the Applicants: elected claims directed to nucleic acids, vectors, and host cells; canceled claims 2, 8-10, 12, and 14-16; amended claims 1, 3, 5, 11, and 13; and added claims 17-25.

In an Office action dated May 28, 1997, claims 1-3, 7, 11, 13, 17-25 were rejected. In an amendment dated November 26, 1997, the Applicants canceled claims 6, 13, and 17; amended claims 1, 3-5, 7, 11, 18, and 20; and added new claims 26-38. In an amendment dated July 23, 1998, the Applicants amend claims 1, 3-5, 7, 18-19, 26-33, and

36-37; and add new claims 39-44. Thereafter, the Patent Office suspended prosecution of the application for approximately 18 months because "a reference relevant to the examination of this application may soon become available."

At the time of issuance of the outstanding Office action, claims 1, 3-5, 7, 11, and 18-44 were pending. In an interview on June 22, 2000, the Examiner requested submission of a renumbered claim set. Thus, the pending claims have been canceled and new claims 45-79 have been substituted therefor. A table correlating old and new claims is set forth for the Examiner's convenience.

Current Claim	Corresponding Old Claim	Comments
Claim 45.	Claim 18.	
Claim 46.	Claim 19.	
Claim 47.	Claim 20.	
Claim 48.	Claim 21.	
Claim 49.	Claim 22.	
Claim 50.	Claim 32.	
Claim 51.	Claim 11.	
Claim 52.	Claim 23.	
Claim 53.	Claim 30.	
Claim 54.	Claim 41.	
Claim 55.	Claim 31.	
Claim 56.	Claim 24.	
Claim 57.	Claim 25.	
Claim 58.	Claim 38.	
Claim 59.	Claim 39.	
Claim 60.	Claim 43.	
Claim 61.	Claim 19.	
Claim 62.	Claim 44.	
Claim 63.	Claim 34.	
Claim 64.	Claim 35.	

Claim 65.	Claim 36.	
Claim 66.	Claim 1.	Additional limitations specifying human polynucleotide and 23 kD polypeptide
Claim 67.	Claim 26.	
Claim 68.	Claim 29.	
Claim 69.	Claim 3.	Additional limitation specifying 23 kD polypeptide
Claim 70.	Claim 4.	
Claim 71.	Claim 5.	
Claim 72.	Claim 40.	
Claim 73.	Claim 7.	
Claim 74.	Claim 42.	Additional limitation specifying human polypeptide
Claim 75.	Claim 37.	

The suspension of this application for more than a year has been detrimental to the Applicants' continued commercial development of this technology, and the Applicants are quite interested in expeditious allowance, now that prosecution has resumed and the "relevant reference" has become available. The claim amendments herein are solely for the purpose of clarity and expediting allowance, and are unnecessary to overcome the Patent Office's rejections. The Applicants reserve the right to pursue the subject matter of claims as originally filed (or later introduced) in subsequent applications, such as continuing applications.

II. The Patent Office's rejection of claims 1, 37, and 42 under 35 U.S.C. §112, first paragraph, for lack of written descriptive support should be withdrawn.

In paragraph 6 of the Office action, the Patent Office rejected claims 1, 37, and 42 under 35 U.S.C. 112, first paragraph, alleging that these claims contain subject matter "which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." Claim 37 was rejected due to its dependence from claim 1. New claims 66 and 74 are analogous to rejected claims 1 and 42.

Although the rejection spans approximately three pages, the Patent Office's principal objections appear to be that the breadth of the claims is such that the claims encompass polynucleotides from other species and polynucleotides which encode variant polypeptides so long as receptor binding activity is maintained. The Patent Office acknowledges the presence of Examples relating to a human Flt4 ligand, and there are no impediments to identifying other human molecules that may be allelic variants, for example. Solely to expedite allowance,¹ the Applicants have included a limitation in claims 66 and 74 to specify a human polynucleotide sequence. The Applicants' teachings of a human sequence, combined with their many additional teachings related to VEGF-C processing, Flt4 binding, Flt4 binding assays, hybridization techniques, and the like, conveys possession of the human genus recited in the claims to a person of ordinary skill. These amendments render moot the rejection for lack of written description, and the rejection should be withdrawn.

III. The Patent Office's rejection of claims 1, 3-5, 7, 11, and 18-44 under 35 U.S.C. §102(e) should be withdrawn.

In paragraph 8 of the Office action, the Patent Office rejected claims 1, 3-5, 7, 11, and 18-44 under 35 U.S.C. §102(e), as allegedly being anticipated by Hu et al. (U.S. Pat. No. 5,935,820). The rejection specifically addressed only claims 11 and 18, although it contained some general reasoning apparently directed at the other claims:

Hu et al. disclose a polynucleotide, SEQ ID NO:1, which encodes a polypeptide, SEQ ID NO:2, which includes a domain defined by 8 cysteine residues of the VEGF family, and which is capable of binding to human Flt4 receptor tyrosine kinase. The instant claims indicate that the polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein, however, this limitation only further defines the processed protein and places no material limitations on the polynucleotide. Claim 11 further defines the polypeptide as comprising amino acids 1 to 120 of SEQ ID NO:33, however, this limitation places no material limitations on the polynucleotide. Claim 18 is directed to a polynucleotide which lacks a

¹ The Applicants reserve the right to dispute the factual and legal premises upon which the rejection is based, and pursue claims of the original or greater scope in continuing applications.

The Applicants also observe that the Hu et al. '820 patent cited in paragraph 7 teaches only a single polynucleotide species yet purports to claim a genus using hybridization claim limitations. See, e.g., Hu et al. claims 41 and 50. The Examiner is requested to clarify the Patent Office's position as to when a single polynucleotide species provides a written description of a hybridization genus that satisfies §112, first paragraph.

portion of the nucleic acid sequence which encodes the cysteine motifs of a Balbiani ring 3 protein, but still encodes a polypeptide that is capable of binding to human Flt4 receptor tyrosine kinase. This limitation appears to be inherently met by the embodiment of claim 1 of '820 in that the mature protein lacks this portion of the polypeptide, therefore, a "polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2" anticipates this claim.

(Office action at p.6.)

The Applicants respectfully traverse.

At the outset, the Applicants wish to clarify certain factual and legal issues raised by the above-quoted rejection. First, the rejection is factually incorrect in that Hu et al. neither discloses nor suggests that any polypeptide binds actually binds to Flt4. In fact, Hu et al. makes no mention of the Flt4 receptor whatsoever, and fails to identify any receptor for "VEGF2" whatsoever. Second, the Applicants object to the Patent Office's suggestion that the scope or wording of the claims of the Hu et al. patent have any relevance to whether Hu et al. is anticipatory under §102(e). The application that matured into the Hu et al. patent was filed on March 27, 1997, more than *two years after* the filing date of the present application, and *after the publication of a PCT application based on the present application* (See WO 97/05250, published February 13, 1997), and *after the publication of the present inventors own work in prominent scientific journals* that would have come to the attention of Hu et al.² Still more of the present inventor's publications were available to Hu et al. in 1997-1999, during prosecution of the Hu et al. application. (See, e.g., Joukov *et al.*, "Proteolytic Processing regulates receptor specificity and activity of VEGF-C," *EMBO J.*, 16(13): 3898-3911 (1997).) The relevant inquiry under §102(e) is the inquiry of what was "described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent." This inquiry requires the Patent Office to ignore what was claimed in the Hu et al. patent, which may have been tainted by knowledge of the present invention, as explained above. The relevant inquiry must focus on what was *described* in those Hu et al. priority applications that have a filing date that could have

² See, e.g., Joukov *et al.*, "A Novel Vascular Endothelial Growth Factor, VEGF-C, Is a Ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) Receptor Tyrosine Kinases," *EMBO J.*, 15(2): 290-298 (1996); and Kukkk E, Lymboussaki A, Taira S, Kaipainen A, Jeltsch M, Joukov V, Alitalo K., "VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development," *Development*, 122(12): 3829-37 (1996).

preceded the invention date of the applicants.³ See, e.g., *In re Benno*, 226 USPQ 683, 686 (Fed. Cir. 1985) ("The scope of a patent's claims determines what infringes the patent; it is no measure of what it discloses. A patent discloses only that which it describes....") The limited teachings of Hu et al. are discussed below.

The Applicants also dispute that the Patent Office's reasoning, even if correct, supports a legitimate anticipation rejection. Independent claims 1, 3, 7, and 42 (now claims 66, 69, 72, and 74) are all directed to host cells that have been transformed or transfected with a nucleic acid and that express an approximately 23 kD polypeptide encoded by the nucleic acid that has particular structural and functional characteristics, such as a particular size or sequence and/or the ability to bind the Flt4 receptor. The Hu et al. patent neither discloses nor suggests the recited polypeptides, or host cells that make such polypeptides, or the activities of the polypeptides. (There is no description in Hu et al. of a 23 kD Flt4 ligand polypeptide or of a host cell that produces such a polypeptide.)

Notwithstanding these claim limitations, the Patent Office rejected the claims. Under the Patent Office's analysis, "this limitation only further defines the processed protein and places no material limitations on the polynucleotide." The Patent Office has apparently ignored the fact that these particular claims are not directed to an isolated polynucleotide, but rather to a host cell that produces a polypeptide having certain characteristics. The Patent Office has failed to explain why a limitation on a novel and nonobvious protein produced by a host cell is insufficient to render novel a claim to the host cell that produces the protein. The Patent Office has apparently ignored the axiom that anticipation of a claim under §102 can be found only if the prior art discloses *every element* of the claim. See, e.g., *In re King*, 801 F.2d 1324, 1326 (Fed. Cir. 1986). An anticipation of a recombinant, protein-producing host cell claim does not exist merely because a polynucleotide has allegedly been described in the prior art.

The Patent Office rejected claim 18 (now claim 45), directed to a polynucleotide, on the basis that "Claim 18 is directed to a polynucleotide which lacks a portion of the nucleic acid sequence which encodes the cysteine motifs of a Balbiani ring 3 protein, but still encodes a polypeptide that is capable of binding to human Flt4 receptor

³ The Applicants reserve the right to dispute whether Hu et al. qualifies as a §102(e) reference, on the grounds that Hu et al. is not a patent granted on an application filed before the invention thereof by the applicant.

tyrosine kinase. This limitation appears to be inherently met by the embodiment of claim 1 of '820 in that the mature protein lacks this portion of the polypeptide, therefore, a 'polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2' anticipates this claim." This reasoning is based on an improper focus on what the cited patent *claimed*, rather than what it *described*. See *In re Benno, supra*. If one reads the Hu et al. patent to determine what Hu et al. actually *describes* as "a polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2" one finds descriptions such as the following:

The polynucleotide of this invention . . . contains an open reading frame encoding a protein of about 350 amino acid residues of which approximately the first 24 amino acid residues are likely to be leader sequence such that the mature protein comprises 326 amino acids.

(Hu et al. at Col. 3, lines 56-63.)

Thus, Hu et al. describes a polynucleotide that encodes the "mature portion of a protein consisting of SEQ ID NO: 2" as a polynucleotide that comprises the final 326 codons of SEQ ID NO: 2.⁴ A study of the approximately 350 codon sequence in Hu et al. (Figures 1-2; SEQ ID NO: 2) shows that the mature protein of 326 amino acids includes the carboxy-terminal domain Balbiani Ring 3 Protein cysteine motifs. Because the "mature protein" described in Hu et al. includes the BR3P domain and falls outside the scope of claim 45, claim 45 is not anticipated. Claim 11 (now 51), which depends indirectly from claim 45, also is not anticipated.

In paragraph 9, the Patent Office acknowledged that certain subject matter was free of the prior art, and in paragraph 8, it suggested that claim 11 might have been an attempt to claim that allowable subject matter, except that its limitation "further defines the polypeptide as comprising amino acids 1 to 120 of SEQ ID NO:33, however, this limitation places no material limitations on the polynucleotide." Claim 11 (now claim 51) is patentable over the art because it depends from claim 45, as explained above. However, the Applicants wish to direct the Patent Office's attention to claim 60 (formerly 43), which claims a nucleic acid and contains the explicit limitation "said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125." The Applicants respectfully submit that claim 60 satisfies the Patent

⁴ The Applicants reserve the right to present evidence that the alleged signal peptide taught in Hu et al. does not operate as a signal peptide at all.

Office's own criteria for allowable subject matter in this case, and should not have been rejected at all.

For the foregoing reasons, the Hu et al. patents neither disclose nor suggest the claimed invention, and the rejections based on Hu et al. under 35 U.S.C. §102(e) should be withdrawn.

IV. Interview Follow-up

During the interview with Examiners Saoud and Kunz and the Applicants' attorneys, the Examiners acknowledged that host cells which produced a fully processed VEGF-C polypeptide of approximately 23 kD were novel and unobvious over the two Hu et al. patents of record, and raised the question of whether this result of the Applicants' was due uniquely to the host cell chosen. The Applicants have filed herewith a declaration to provide evidence that they have succeeded in producing a Flt4 ligand of approximately 23 kD in several other host cells. An executed version of the declaration will be submitted under separate cover.

V. Information Disclosure

On March 21, 2000, the Patent Office issued a third patent to Hu et al., U.S. patent No. 6,040,157. The '157 patent is a CIP that was filed in December, 1997, after many publications by the present applicants and after the present application was filed. The Applicants wish to draw the Examiner's attention to the '157 patent. For §102(e) purposes, the '157 patent is cumulative to the '540 and '820 patents of record. (To the extent it is not cumulative in disclosure, it is not citable as *prima facie* prior art, because the non-cumulative disclosure is not entitled a date that precedes the January 12, 1996, filing date of the present application.)

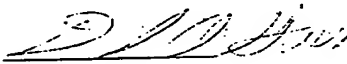
VI. Summary

The Applicants respectfully request entry of the foregoing amendments and allowance of all of the pending claims in view of the foregoing remarks.

Respectfully submitted,

MARSHALL, OTOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

Dated: August 4, 2000


David A. Gass
Registration No. 38,153



RECEIVED

AUG 13 2000

PATENT

1646

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.) Title: RECEPTOR LIGAND
Serial No: 08/585,895) Group Art Unit: 1646
Filed: January 12, 1996) Examiner: Christine Saoud

TRANSMITTAL LETTER

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an executed declaration of Kari Alitalo for entry into the file for the above-identified matter. An unsigned version of this declaration was previously filed, together with an amendment, on August 4, 2000. The Applicants believe that this declaration should be entered without petition or fee for additional extension of time, because a fully responsive amendment to the Office action of April 4, 2000, has already been filed. However, if extension of time is required, please consider this transmittal to be a request therefor, and charge an additional extension fee to deposit account No. 13-2855.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 10, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass



RECEIVED

AUG 18 2000

PATENT

TECH CENTER 1600/2500

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)	Title: RECEPTOR LIGAND
Serial No: 08/585,895)	Group Art Unit: 1646
Filed: January 12, 1996)	Examiner: Christine Saoud

TRANSMITTAL LETTER

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an executed declaration of Kari Alitalo for entry into the file for the above-identified matter. An unsigned version of this declaration was previously filed, together with an amendment, on August 4, 2000. The Applicants believe that this declaration should be entered without petition or fee for additional extension of time, because a fully responsive amendment to the Office action of April 4, 2000, has already been filed. However, if extension of time is required, please consider this transmittal to be a request therefor, and charge an additional extension fee to deposit account No. 13-2855.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 10, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass



#3/
12.7

PATENT
Attorney Docket No. 28967/33072
LUD 5453.1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitalo et al.

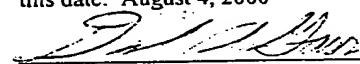
Serial No.: 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Group Art Unit: 1646

Examiner: SAOUD, Christine

) I hereby certify that this paper and the
) documents referred to as enclosed
) herewith are being deposited with the
) United States Postal Service as First Class
) Mail, postage prepaid, in an envelope
) addressed to: Assistant Commissioner
) for Patents, Washington, DC 20231, on
) this date: August 4, 2000
) 
) David A. Gass
) Reg. No.: 38,153
) Attorney for Applicants
)

Declaration Pursuant to 37 C.F.R. § 1.132 of Kari Alitalo

I, Dr. Kari Alitalo, declare and state as follows:

Introduction

1. I am a co-inventor of the subject matter of the above-identified patent application (hereinafter "the patent application"). I make this declaration to provide evidence to the Patent Office that may be relevant to the patentability of pending claims. Specifically, some of the pending claims in the application relate to recombinant host cells that produce a mature Flt4 receptor ligand polypeptide of approximately 23 kD (as assessed by SDS-PAGE under reducing conditions). This declaration is intended to provide evidence and confirmation that we have achieved expression of this mature form using a variety of host cells transformed/transfected with a full length cDNA encoding a 419 residue prepro-form of the ligand.

Evidence

I. Introduction

2. My co-inventor Dr. Joukov and I (together with others in my laboratory) have conducted substantial experiments to evaluate the proteolytic processing of human prepro-VEGF-C, a protein of 419 amino acids, into a mature form which has undergone substantial N-terminal and C-terminal processing. The results of many of the VEGF-C processing experiments are succinctly and accurately reported in our publication Joukov *et al.*, "Proteolytic processing regulates receptor specificity and activity of VEGF-C," *EMBO J.*, 16(13): 3898-3911 (1997). I hereby reaffirm the accuracy of the data reported in that paper, which I incorporate by reference, and summarize only briefly in the next paragraphs of the introduction.

3. The VEGF-C gene encodes a mRNA for the synthesis of a prepro-protein Flt4 ligand precursor of 419 amino acids. (The complete 419 codon cDNA was deposited with the ATCC and is cross-referenced at pages 6, 28-29, and 39 of the patent application, and its sequence is deposited as SEQ ID NOs: 44 and 45 in the Sequence Listing.) The "pre-pro-protein" is processed to remove a signal peptide and two pro-peptides to produce a fully mature, most active form of VEGF-C. Initially, the "pre" part or signal sequence is cleaved off upon its translocation through the cellular membrane in the rough endoplasmic reticulum of the synthesizing cells. The rest of the polypeptide is then translocated across the cell membrane upon its continued elongation synthesis.

4. A proteolytic cleavage to cleave a C-terminal pro-peptide occurs preferentially between amino acid residues 227 and 228, separating the N-terminal and C-terminal halves (roughly) of the constituent polypeptides.¹ This polypeptide, once cleaved in the middle, is

¹ The C-terminal half contains cysteine residue repeat patterns reminiscent of the Balbiani Ring 3 Protein (BR3P). The N-terminal half contains a series of cysteine residues in a pattern shared with other members of VEGF/PDGF family.

Position 227 of the 419 codon sequence corresponds to position 125 of SEQ ID NO: 33, the sequence referred to in the claims of the patent application.

only partially active relative to the fully mature, proteolytically processed VEGF-C that is created upon removal of the N-terminal pro-peptide from the N-terminal half.

5. Another cleavage of the VEGF-C protein precursor to remove an N-terminal pro-peptide occurs on the amino-terminal side of the domain that shares a cysteine motif in common with other members of the VEGF-PDGF family. In our experiments we have observed that this second pro-peptide cleavage occurs in at least two preferential peptide bonds: one is between amino acid residues 102 and 103,² and the other one between residues 111 and 112. These two alternative cleavages that remove the N-terminal pro-peptide produce fully processed forms of the Flt4 ligand which have a similar potency of high affinity binding to KDR/VEGFR-2, which is expressed in blood vascular endothelium and lymphatic endothelium and to Flt4/VEGFR-3, which is predominantly expressed in the lymphatic endothelium. The fully processed mature forms of VEGF-C that are most active have a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

II. Recombinant Host cells that produce a fully processed VEGF-C of about 23 kD

6. Several of our initial experiments were done using 293EBNA or 293 T-cells as host cell systems for the transfection of VEGF-C expression vectors. These cells were fairly efficient in the processing of the prepro-VEGF-C into the fully processed ~23 kD form. In the initial experiments described in our patent application, VEGF-C was eluted from

² Position 103 (Threonine) of the 419 codon sequence corresponds with position 1 of SEQ ID NO: 33, the sequence referred to in the claims of the patent application.

an Flt4-EC affinity matrix using pH 2.4, which seemed to enhance (but was not necessary for) the proteolytic processing into the mature form.³

7. We have also observed similar proteolytic processing in COS monkey cells and in the HT1080 human fibrosarcoma cells. Experimental details with COS and HT1080 cells are reported in Joukov *et al.* (1997), *supra*. The cells were grown in a conventional commercial media (Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum.⁴ The vector used for transformation of these cells was a pREP7 vector containing the 419 codon VEGF-C cDNA, essentially as described in the patent application. Processing to produce the ~ 23 kD form was observed in both COS and HT1080 cells, although it was significantly less efficient than in the 293 cells.

8. We have also used the MCF-7 breast carcinoma cell line to produce the ~23 kD mature form of VEGF-C. Unlike the 293 or COS cells, MCF-7 cells do not replicate the transfected plasmid. MCF-7 cells were transfected with pEBS7 expression vector⁵ containing a cDNA insert coding for human prepro-VEGF-C cDNA (419 codon form) or with an empty vector, and stable cell pools were selected. The transfected MCF-7 cells were grown in RPMI-1640 medium (a commercially available medium) containing 10% FCS and 150 µg/ml Hygromycin B. To study forms of VEGF-C produced, the cells were metabolically labeled,

³ Like all enzymatic reactions, the proteolytic cleavages in the VEGF-C polypeptide backbone occur in an enzyme- and substrate concentration- dependent manner, and can be influenced by factors such as pH, time, and protease inhibitors. Our data suggests that the enzymes that cleave the N-terminal pro-peptide from pro-VEGF-C are secreted from both VEGF-C-producing and from other cell types. We also have observed increased processing upon the depletion of the culture medium of fetal calf serum.

⁴ By way of comparison, Example 13 of the patent application describes culturing 293-EBNA cells in DMEM-0.2% BSA. As explained in the previous footnote, lower serum levels in the media appears to correlate with increased processing.

⁵ The pEBS7 expression vector was known in the literature at least as early as 1991, years before the priority date of the application. (See, e.g., Peterson, C. and Legerski, R., "High-frequency transfection of human repair-deficient cell lines by an Epstein Barr virus-based cDNA expression vector," *Gene*, 107(2): 279-284 (1991). We selected pEBS7 because this vector promotes high expression levels in MCF-7 cells. The vector contains a CMV promoter and hygromycin B and ampicillin resistance genes.

and after 96 hours, VEGF-C was bound to an Flt4 affinity substrate.⁶ The bound proteins were analyzed in 12.5% SDS-PAGE under reducing conditions. As can be seen from Figure A, the VEGF-C in the culture medium that bound to the soluble receptor Flt4-Ig affinity substrate consists of both the 29/31 kD and ~23 kD forms. Thus, expression of VEGF-C protein in these cells occurs in smaller quantities than other cells we have tested which replicate the transfected plasmid, yet we can recover both the 31/29 and ~23 kD forms of VEGF-C from the culture medium of these cells using the type of affinity matrix described in the patent application.

9. We also have achieved production of the ~23 kD form of VEGF-C using the baculovirus expression system in insect cells. In one set of the experiments, 3 million Sf-9 cells each were infected with baculoviral clones 32/1-32/5 and 34/1-34/5 expressing full length (419 codon) untagged hVEGF-C under the polyhedrin promoter.⁷ Seven days post-infection, the supernatant was harvested and the remaining cells lysed in 350 µl RIPA buffer. Ten microliters of supernatant of clone 32/1 and 5 µl lysate of clones 32/1 - 32/5 and 34/1 - 34/5 were subjected to 15% SDS PAGE, and VEGF-C specific bands were immunodetected after Western blotting using antiserum raised against VEGF-C peptide (residues 104-120, or

⁶ The affinity substrate that we used was Flt4(1-3)Fc, which comprises the three immunoglobulin-like regions of the Flt4 extracellular domain which have been shown to be responsible for ligand binding. Thus, this affinity matrix is the functional equivalent of the Flt4-EC affinity matrix described in the patent application.

⁷ The cell line Sf9 is a clonal isolate of Sf21 cells, which are derived from ovary cells of the fall army worm, *Spodoptera frugiperda*. The cell line was maintained as adherent culture at 27°C in TMN-FH media completed with fetal bovine serum to a final concentration of 10%. In addition, 100 mg/ml streptomycin and 10 units/ml penicillin were used to minimize the risk of bacterial contamination. The cells were cultured using routine and standard procedures for these experiments. (See, e.g., O'Reilly et al., *Baculovirus Expression Vectors: a laboratory manual*. W.H. Freeman and Company. New York, 1992: pp. 109-122).

For virus production and amplification, 5 baculoviral clones (1-5) were purified from two transfection supernatants (32 and 34), that were obtained using the FASTBAC system (GIBCO/Life Technologies) according to the instructions of the manufacturer. Transfections 32 and 34 were performed using two bacmid DNA preparations from independently obtained clones using shuttle vector pFB1-hVEGF-C-FL. Stock virus was obtained by two rounds of amplification after plaque purification. For the first amplification, 2.5 Mio. Sf-9 cells were inoculated with the whole purified plaque and incubated for 5 days. For the second amplification 8 Mio. Sf-9 cells were inoculated with 1/40 of the total virus obtained in the first amplification step and incubated for 5 days.

2-18 of the mature form). The results, depicted in Figure B, show clearly that the major form of VEGF-C in the lysates of 7 day p.i. cells is the 21/23 kD form. Uncleaved (prominent band), 29/31 kDa (prominent band), and ~23 kDa forms (weaker band) were present in the supernatant. These experiments show that insect cells also cleave the VEGF-C protein to the ~23 kD mature form and that this insect cell expression system can provide a source for large-scale production of the ~23 kD form of the protein.

10. In another series of experiments, we transfected the MeWo cell line,⁸ established from a lymph node metastasis of a nodular malignant melanoma, to constitutively overexpress a prepro-VEGF-C cDNA (419 codon form)⁹. Ordinary commercial media was employed for these experiments also (RPMI 1640 medium with 5% fetal bovine serum (FBS), purchased from Gibco BRL, Grand Island, NY). As determined by Northern analysis, the parental MeWo cell line and three vector-transfected control clones (MeWo/control) did not express any detectable amounts of VEGF-C mRNA *in vitro* or *in vivo*. Three VEGF-C transfected cell clones (MeWo/VEGF-C) expressed high levels of VEGF-C mRNA in culture, as well as in tumors (when introduced into mice) that reached the size of ~1200 mm³. Western blot analyses using antibodies raised against a VEGF-C peptide confirmed that high VEGF-C mRNA levels correlated with high amounts of VEGF-C protein expression. We

⁸ The human malignant melanoma cell line MeWo (Sordat, B.C. M., Y. Ueyama, and J. Fogh. 1982. Metastases of tumor xenografts in the nude mouse. In *The nude mouse in experimental and clinical research*. J. Fogh, and B.C. Giovanella, editors. Academic Press, New York. 95-147; Kerbel, R.S., M.S. Man, and D. Dexter. 1984. A model of human cancer metastasis: extensive spontaneous and artificial metastasis of a human pigmented melanoma and derived variant sublines in nude mice. *J Natl Cancer Inst.* 72:93-108), kindly provided by Dr. Robert S. Kerbel (Sunnybrook Health Science Centre, Toronto, Canada)

⁹ A 1997 bp full-length (419 codon) human VEGF-C cDNA (GenBank accession number X94216) was cloned into a pcDNA3.1/Zeo expression vector (Invitrogen, San Diego, CA) which contains a CMV-enhancer-promoter and a Zeocin selection cassette. The sequence and the orientation of the VEGF-C gene in the construct were verified by restriction mapping and by direct sequencing using the Sanger dideoxy method. Subconfluent cell cultures were transfected either with pcDNA3.1/Zeo vector containing the full-length human VEGF-C cDNA in sense orientation or with the vector alone using the Superfect transfection reagent (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. Forty-eight hours after transfection, cells were split 1:5 into their full growth medium containing 50 mg/ml Zeocin (Invitrogen) to select transfectants. Stably transfected cell clones were individually expanded and analyzed for VEGF-C mRNA expression and protein secretion.

detected a strong band of approximately 60 kDa in cell lysates of VEGF-C transfectants, corresponding to VEGF-C precursor, and only trace amounts in control cells. Large amounts of the secreted 31 kDa form were observed in culture supernatants of VEGF-C transfected clones, whereas the secreted protein was not detectable in supernatants of control cells. The mature ~23 kDa VEGF-C form was detected in tumor lysates.

11. The foregoing experiments demonstrate that we were able to recombinantly express the ~23 kD mature form of the F1t4 ligand VEGF-C in a variety of human cell lines transfected with a 419 codon prepro-VEGF-C cDNA, as well as in a Cos monkey cell line and an insect cell line.

Certification

12. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

Kari Alitalo

Date: _____

Figure A

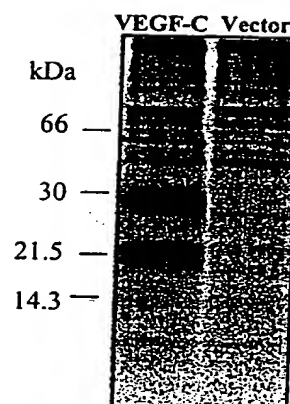
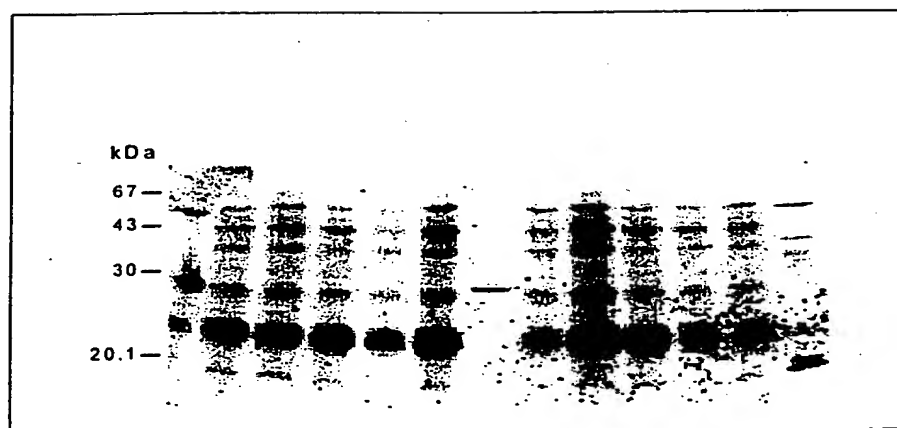


Figure B



38/5
D12
2/27
PATENT

Attorney Docket No.: 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.

Serial No: 08/585,895

Filed: January 12, 1996

Title: RECEPTOR LIGAND

Allowed: October 24, 2000

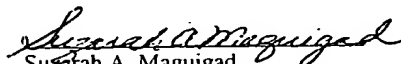
Batch No.: U18

Group Art Unit: 1647

Examiner: C. Saoud

) I hereby certify that this paper is being
) deposited with the United States Postal
) Service, in an envelope addressed to the:
) Commissioner for Patents, Box Issue
) Fee, Washington, D.C. 20231, utilizing
) the "Express Mail Post Office" under
) Mailing Label No. EL566464161US on
) this date:

) January 24, 2001

) 
) Suzafah A. Maguigad



AMENDMENT AFTER ALLOWANCE PURSUANT 37 C.F.R. § 1.312

Commissioner for Patents
Box Issue Fee
Washington, D.C. 20231

Dear Sir:

Please amend this application as follows:

AMENDMENTS

In the Specification:

At page 1, line 3, after "August 1, 1995.", please delete the following priority claim, which was introduced by way of Amendment filed July 23, 1998:

"This application is also a continuation-in-part of U.S. Patent Application Serial No.

08/340,011, filed November 14, 1994, now U.S. Patent No. 5,776,755."

At page 8, line 7, please delete "Figure 2 schematically depicts" and insert --
Figures 2A and 2B schematically depict--.

At page 8, line 29, please delete "Figure 9B shows" and insert --Figures 9B-D
show--.

At page 8, line 30, please delete "Figures 10A-10B" which was introduced by
way of Amendment filed November 26, 1997 and insert --Figures 10A-D--.

At page 14, line 32, please delete "Figure 2" and insert --Figures 2A and 2B--.

At page 27, line 30, please delete "Figure 9B" and insert --Figures 9B through
9D--.

At page 28, line 1, please delete "Figure 10" and insert --Figures 10A through
10D--.

At page 28, line 6, please delete "Fig. 9B" and insert --Figures 9B through
9D--.

At page 29, line 13, please delete "Fig. 10" and insert --Figures 10B and
10C--.

REMARKS

Applicants request entry of the foregoing amendments, which relate solely to
formal matters. These amendments are being presented prior to or concurrently with payment
of the issue fee as required by Rule 312. The amendments do not affect the scope or content of
the allowed claims. The Patent Office is authorized to charge any fee associated with this
amendment to Deposit Account No. 13-2855.

The amendment to page 1 amounts to a cancellation of a priority claim to an

application that was filed in November, 1994. The Applicants continue to maintain their priority claim to U.S.S.N. 08/510,133, filed August 1, 1995, as stated in the application as originally filed. The sole purpose behind cancellation of the 1994 priority claim is to maximize patent term of the eventual patent, because it is the Applicants' understanding of current law that the term of this patent will be measured from the earliest claimed priority date. The priority claim cancellation is not intended as an admission of whether or not the claimed invention would be entitled to priority, if the priority claim to the November, 1994 application were maintained. The Applicants reserve the right to maintain the same priority claim for subject matter that may be pursued in related applications, such as continuations, continuations-in-part, divisional applications, reissue applications, or the like. It is the Applicants' understanding from prosecution that the subject matter of the allowed claims has been deemed patentably distinct from any subject matter disclosed in art of record, including subject matter disclosed in U.S. patent issued to Human Genome Sciences (Hu et al., U.S. Patent No. 5,935,820) that was considered by the Examiner. (This patent was cited by the Examiner as a reference under §102(e) and distinguished by the Applicants. See Amendment dated August 4, 2000, at pages 11-15.) Thus, the presence or absence of the priority claim raises no patentability issues.¹

The remaining amendments to the specification merely conform the specification to the formal drawings submitted concurrently herewith. Figures 2, 5, 9 and 10 were prepared

¹ The November, 1994 patent application has issued as U.S. Patent No. 5,776,755. The '755 patent is not prior art under §102(e) because, to the extent the '755 patent discloses or suggests the present invention, the relevant disclosure is a disclosure of the present inventors' own work. Because the relevant portions of the '755 patent constitute the inventor's own work, the relevant filing date of the '755 patent was not "before the invention thereof by the applicant" as required by §102(e). (It is impossible to disclose the inventors' own work before the inventors invented it.)

on multiple sheets and/or renumbered in order to comply with the Draftsman's requirements. The specification has been amended to reflect the fact that these figures will be multiple pages in the issued patent.

These amendment add no new matter and do not raise any new patentability issues that would require any substantive examination by the Examiner.

In view of the foregoing, the applicant respectfully requests the granting of the amendment after allowance.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By:



David A. Gass
Registration No. 38,153
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606

January 24, 2001

Allowed: October 24, 2000
Batch No.: U18
Application No.: 08/585,895

1. Small Entity Status

- ☐ Verified statement(s) claiming small entity status is(are) attached.
☒ Small entity status has been established and is still effective.
☐ Has not been established.

2. Deposit Account and Refund Authorization

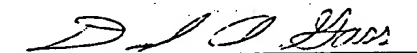
- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.
☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

January 24, 2001

By:



David A. Gass
Reg. No: 38,153



Allowed: October 24, 2000
Batch No.: U18
Application No.: 08/585,895

PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.)	Allowed: October 24, 2000
)	Batch No.: U18
Serial No: 08/585,895)	Application No.: 08/585,895
)	
Filed: January 12, 1996)	Title: RECEPTOR LIGAND
)	
)	Group Art Unit: 1647
)	
)	Examiner: C. Saoud

TRANSMITTAL LETTER

Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are the following for entry in the above-identified case:

1. Amendment After Allowance;
2. Thirty sheets of formal drawings (Figs. 1, 2A-2B, 3-4, 5A-5C, 6-8, 9A-9D, 10A-10D, 11-12, 13A-13B, 14A-14B, 15A-15B, 16A-16B, 17-18); and
3. Request for correction of Drawing with sketch showing proposed change to Figure.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper is being deposited with the United States Postal Service, in an envelope addressed to the: Commissioner for Patents, Box Issue Fee, Washington, D.C. 20231, utilizing the "Express Mail Post Office" under Mailing Label No. EL566464161US on January 24, 2001.

Suzanne A. Maguigad
Suzanne A. Maguigad

Allowed: October 24, 2000
Batch No.: U18
Application No.: 08/585,895

1. Small Entity Status

- ☐ Verified statement(s) claiming small entity status is(are) attached.
☒ Small entity status has been established and is still effective.
☐ Has not been established.

2. Deposit Account and Refund Authorization


- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.
☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

January 24, 2001

By:

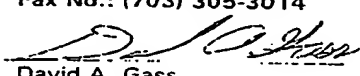

David A. Gass
Reg. No: 38,153

Nov. 2. 2000 3:40PM MARSHALL, O'TOOLE

No. 5291 P. 2/2
From: 0819

PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)	I hereby certify that this paper is
Serial No: 08/585,895)	being sent via facsimile to:
Filed: January 12, 1996)	Commissioner for Patents,
Title: Receptor Ligand)	Washington, D.C., 20231 on this
Group Art Unit: 1646)	date: Date: November 2, 2000.
Examiner: Christine Saoud)	Fax No.: (703) 305-3014
)	
)	David A. Gass
)	Registration No. 38,153
)	Attorney for Applicants

Commissioner for Patents
Washington, D.C. 20231

CHANGE OF ADDRESS

Sir:

The undersigned is an attorney of record in this case. Please mail all correspondence in this case to the undersigned at the address below :

David A. Gass
Marshall, O'Toole, Gerstein, Murray & Borun
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402

The attorney's phone number is (312) 474-6300.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

Dated: November 2, 2000


David A. Gass
Registration No. 38,153

OK to Enter

REMARKS

FIG. 9B illustrates the nucleotide and deduced amino acid sequence of the coding portion of Flt4 ligand cDNA, in which the cleavage site for the putative signal peptide is indicated with a shaded triangle, as disclosed in the specification at page 8, lines 29-31.

The drawing change is being made solely to correct the location of the shaded triangle which indicates the cleavage site demarking a mature VEGF-C protein. Particularly, the shaded triangle should be positioned between "Arg" and "Thr" and not "Ser and Arg". The position of the shaded triangle indicates the start of the designation of the portions of SEQ ID NO: 33 which correspond to the "mature" forms of VEGF-C. The change finds support as originally filed because the description of the amino terminus of a mature form of VEGF-C is found in the specification at p. 23, lines 5-10, and is confirmed at page 25, line 27 to page 26, line 6 (from which it is apparent that the first 13 amino acid residues of a secreted Flt4 ligand are encoded by the thirty-nine 3' bases of SEQ ID NO: 25 that begin ACAGAAGAGACT...). Similar changes to the numbering of residues in the Sequence listing were made in an Amendment filed by the Applicants on November 26, 1997, and were approved by the Examiner. The changes made herein are consistent with what was earlier done the prosecution of this application.

In view of the foregoing, it is submitted that the change to FIG. 9B does not introduce new matter into the disclosure of the application or to the drawings. Accordingly, applicants request approval of the above drawing change.

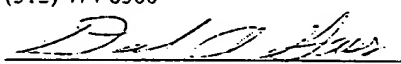
Corrected formal drawings will be provided for the above-identified
application.

Respectfully submitted,

MARSHALL, OTOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

January 24, 2001

By:


David A. Gass
Registration No. 38,153
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606

MetThrValLeuTyrProGluTyr
 CAGCACTACCCCTCTCTCTCCAGCTGACATGACTCATGCTGACTCTACTCTACCCCAATAT
 10 20 30 40 50
 TrpLysMetTyrLysCysGlnLeuArgLysGlyGlyTrpGlnHisAsnArgGluGlnAla
 TCGAAAAATGTACAGTGTACCTTAAGGAAAGAGGCTGCAACATAACAGACAGAGCC
 60 70 80 90 100
 AsnLeuAsnSerArgThrGluGluThrIleLysPheAlaAlaAlaHisTyrAsnThrGlu
 AACCTCACTCAACGACAGACAGACTATAAAATTCTCTGACACATTATAACACAG
 110 120 130 140 150
 IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys
 ATCTTGAAAGCTATTGATAATGACTGCAAGAACTCAATGCATGCCACGGAGCTGTCT
 160 170 180 190 200
 IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProProCysVal
 ATAGATGTCGCAAGGAGTTTGGAGTCCGACAAACACCTTCTTTAAACCTCCATGTCTG
 210 220 230 240 250
 SerValTyrArgCysGlyGlyCysCysAsnSerGluGlyLeuGlnCysMetAsnThrSer
 TCCCTCTACAGATGTCGCGTCTGCTGCAATAGTCCAGGGCTGCAATGCATCAACACCG
 260 270 280 290 300
 ThrSerTyrLeuSerLysThrLeuPheGluIleThrValProLeuSerGlnGlyProLys
 ACGAGCTACCTCAGCAAGCTTATTTCALATTACAGTCCCTCTCTCTCAAGCCCCCAA
 310 320 330 340 350
 ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal
 CCAATACAACTGATTTTGGCAATCAGACTTCTGCGATGCCATGCTCTAAACGCTATCT
 360 370 380 390 400
 TyrArgGlnValHisSerIleIleArgArgSerLeuProAlaThrLeuProGlnCysGln
 TACAGACAGTTTCAATTCATTATAGAGCTTCCCTGCCAGCACACTACACAGTGTCTG
 410 420 430 440 450
 AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu
 GCAGCGAACAGACCTGCCCCACCAATTACATGCGAATATACATCTGCGATGCTCTG
 460 470 480 490 500
 AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspAspSerThrAspGlyPheHis
 CCTCAGCAAGATTTTATGTTTCTCTGCGATGCTGCGATGCTCAACACATGCTATCCAT
 510 520 530 540 550
 AspIleCysGlyProAsnLysGluLeuAspGluGluThrCysGlnCysValCysArgAla
 CACATCTGTCGACCAACAGAGGCTGCGATGAGAGACCTGTCAGTGTCTGTCAGAGCG
 560 570 580 590 600
 GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys
 GGGCTTCCGCTTCCAGCTGTGCGACCCCAAGAACTAGACAGAACTCATGCGCTGT
 610 620 630 640 650
 ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAsn
 CTCCTGTAACAAACTCTTCCCAAGCAATGTGGGCCCAACCGAATTTGATCAAAAC
 660 670 680 690 700
 ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys
 ACATGCCAGTGTGTATGTAAGAACCTGCCCCAGAAATCAACCCCTAAATCCTGCAAAA
 710 720 730 740 750
 CysAlaCysGluCysThrGluSerProGlnLysCysLeuLeuLysGlyLysLysPheHis
 TGTGCTGTGTAATGTACAGAAAGTCCACAGAAATGCTGTGTAAGAAAGAAAGAGTTCAC
 760 770 780 790 800
 HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro
 CACCAACATCCAGCTGTTACAGAGCGCTTACAGAACCCGCAAGGCTTGTGAGCCA
 810 820 830 840 850
 GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln
 GGAATTTTATATCTCAAGAACTGTCTGCTTCTGCTGCTTCTATTTGGAAGAGACCAAA
 860 870 880 890 900
 MetSerEnd
 ATGAGCTAAGATGCTACTGTTTCCAGTTCATGATTTCTATATGCAAAACTGTCTG
 910 920 930 940 950
 1090 1110 1130

FIG. 9B

APPROVED:
 DRAFTING:
 FIG.
 SUBCLASS

1 / 30

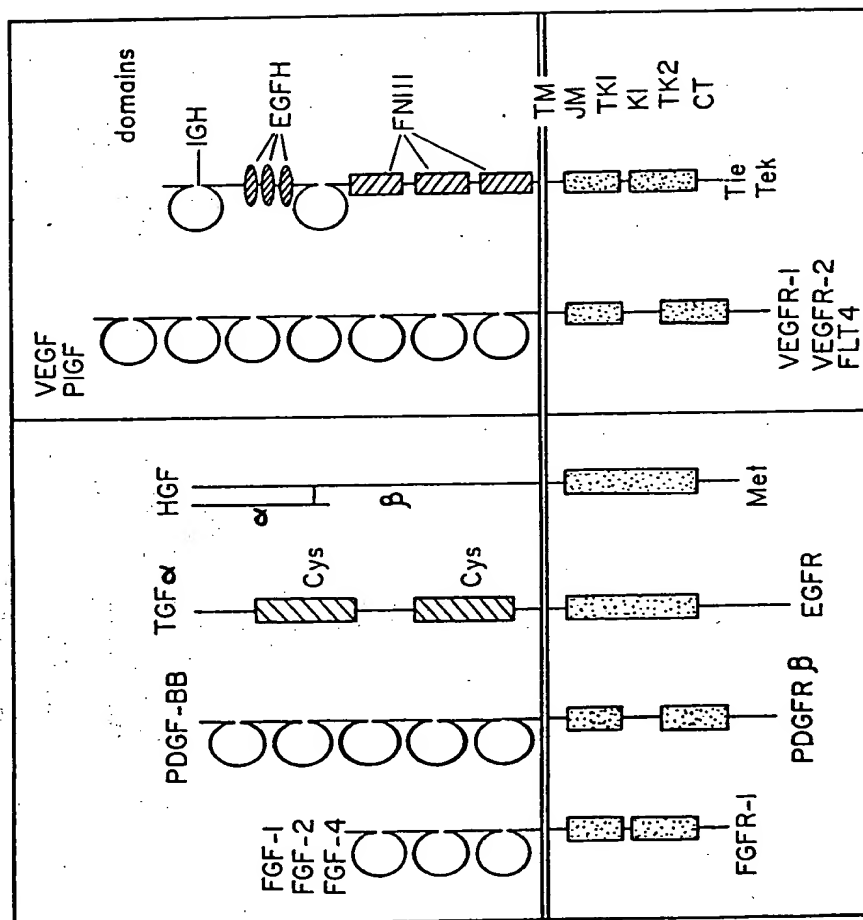
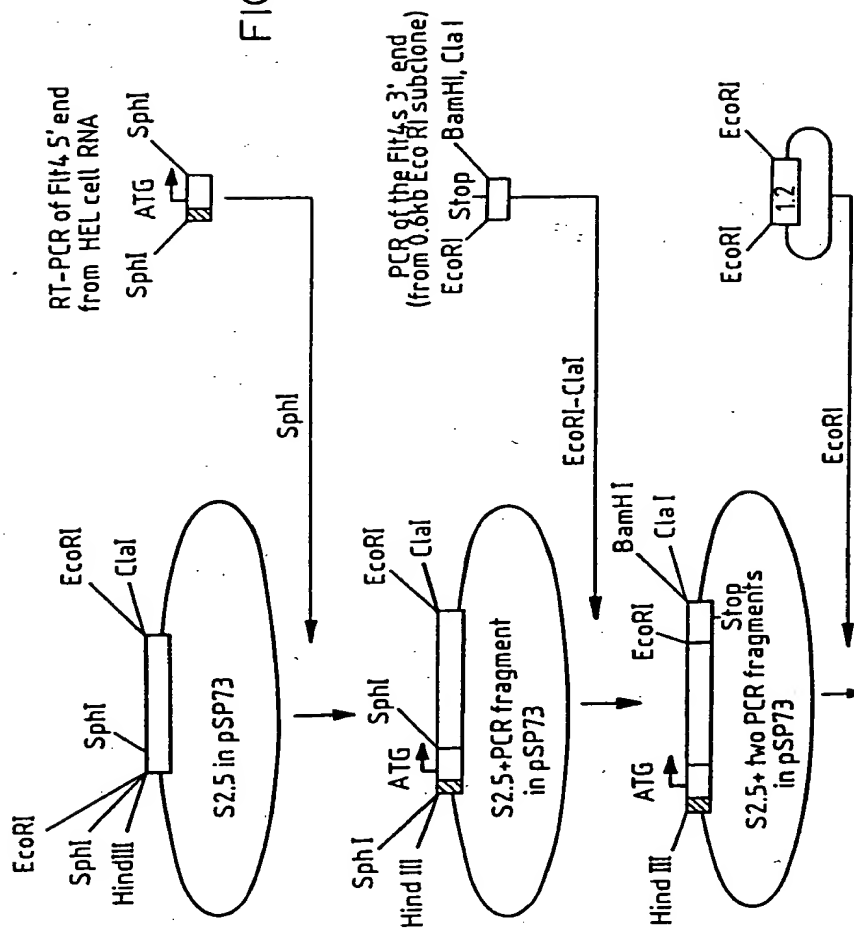


FIGURE 1

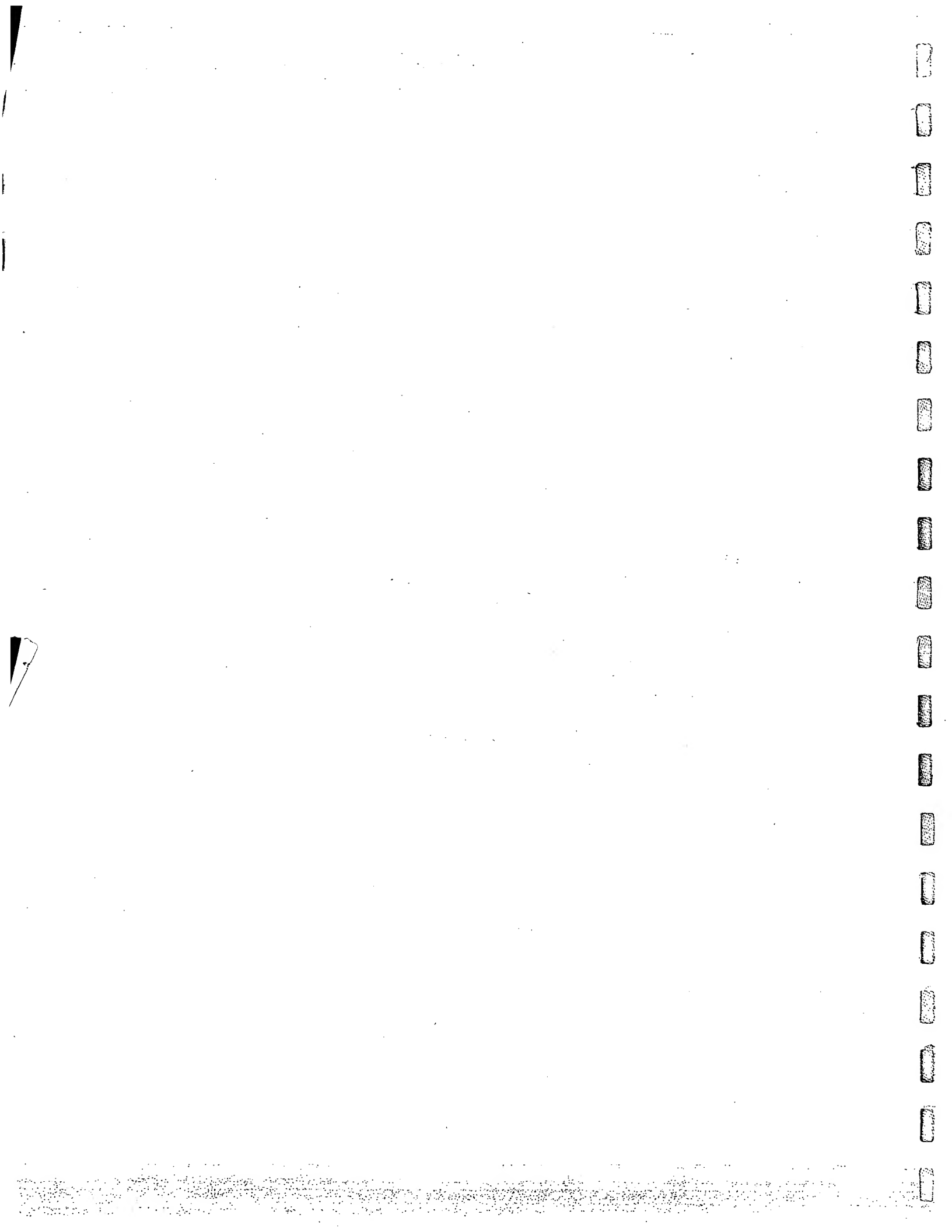
FIGURE 2A

2 / 30

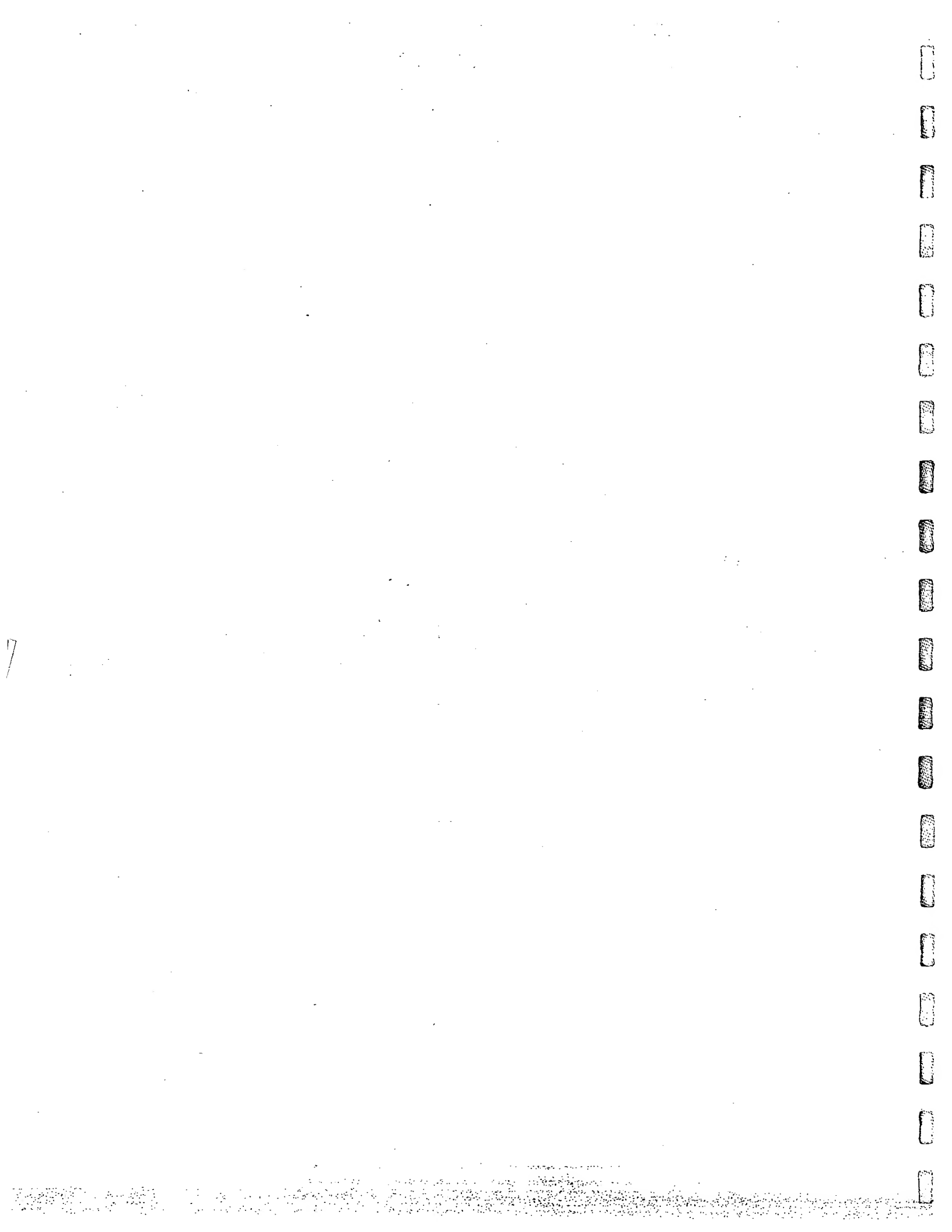


File History
U.S. Patent No. 6,221,839
Issued April 24, 2001

1. Application as filed
8/1/95
2. Information Disclosure Statement
11/3/95
3. Notice to File Missing Parts
11/20/95
4. Response to Notice to File Missing Parts
12/19/95
5. Restriction Requirement
5/29/96
6. Election with Traverse in Response to Restriction Requirement
7/24/96
7. Preliminary Amendment
8/12/96
8. Office Action
9/10/96
9. Request for Amendment of Drawing
2/10/97
10. Amendment and Reply Pursuant to 37 C.F.R. §1.111 and 1.115
2/10/97
11. Statement Claiming Small Entity Status
3/27/97
12. Information Disclosure Statement
4/10/97
13. Final Office Action
4/11/97



14. Amendment After Final Action
6/11/97
15. Suspension of Prosecution
6/24/97
16. Status Inquiry
12/29/97
17. Transmittal of Powers of Attorney
2/24/98
18. Communication in Response to Status Inquiry
4/3/98
19. Supplemental Information Disclosure Statement
10/26/99
20. Office Action
4/26/00
21. Office Action
6/29/00
22. Amendment and Reply Pursuant to 37 C.F. R. 1.111
7/24/00
23. Amendment and Statement Pursuant to 37 C.F.R. 1.825
8/22/00
24. Notice of Allowability
25. Notice of Allowance and Issue Fee Due
10/3/00
26. Change of Address
11/2/00
27. Amendment After Allowance
1/3/01
28. Transmittal of Formal Drawings/Issue Fee Transmittal
1/3/01





US006221839B1

(12) United States Patent
Alitalo et al.**(10) Patent No.: US 6,221,839 B1**
(45) Date of Patent: Apr. 24, 2001**(54) FLT4 LIGAND AND METHODS OF USE****(75) Inventors:** Kari Alitalo, Espoo; Vladimir Joukov, Helsinki, both of (FI)**(73) Assignees:** Helsinki University Licensing Ltd. Oy, Helsinki (FI); Ludwig Institute for Cancer Research, New York**(*) Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.**(21) Appl. No.:** 08/510,133**(22) Filed:** Aug. 1, 1995**(51) Int. Cl.⁷** A61K 38/18; C07K 14/475**(52) U.S. Cl.** 514/12; 514/2; 530/399**(58) Field of Search** 530/399; 514/2; 514/21**(56) References Cited****U.S. PATENT DOCUMENTS**

5,219,739	•	6/1993	Tischer et al.	435/69.4
5,326,695	•	7/1994	Andersson et al.	
5,332,671	•	7/1994	Ferrara et al.	435/240.1
5,932,540	•	8/1999	Hu et al.	
5,935,820	•	8/1999	Hu et al.	

FOREIGN PATENT DOCUMENTS

0 506 477 A1	•	3/1992	(EP)
9524473	•	9/1995	(WO)
WO 95/33050			
A1		12/1995	(WO)
WO 95/33772		12/1995	(WO)
WO 96/11269			
A2		4/1996	(WO)
WO 96/30046			
A1		10/1996	(WO)
WO 96/39421			
A1		12/1996	(WO)
WO 96/39515			
A1		12/1996	(WO)
97/05250		2/1997	(WO)
97/09427		3/1997	(WO)
97/17442		5/1997	(WO)

OTHER PUBLICATIONS

Sitaras et al. Constitutive production of platelet-derived growth factor-like proteins by human prostate carcinoma cell lines. *Cancer Research*, vol. 48, No. 7, pp. 1930-1935, Apr. 1, 1988.*

Fournier et al. Mutation at tyrosine residue 1337 abrogates transforming capacity of the FLT4 receptor. *Oncogene*, vol. 11, No. 3, pp. 921-931, Sep. 7, 1995.*

Pajusila et al. Signalling properties of FLT4, a proteolytically processed receptor tyrosine kinase related to two VEGF receptors. *Oncogene*, vol. 9, No. 12, pp. 3545-3555.*

Galland et al. The FLT4 gene encodes a transmembrane tyrosine kinase related to the vascular endothelial growth factor receptor. *Oncogene*, vol. 8, No. 5, pp. 1233-1240, May 1993.*

U.S. application No. 08/207,550, Jing-Shan Hu et al., filed Mar. 8, 1994.

U.S. application No. 08/465,968, Crain Rosen et al., filed Jun. 6, 1995.

U.S. application No. 60/003,491, James Lee et al., filed Sep. 8, 1995.

U.S. application No. 08/554,374, Lyman, S., filed Nov. 8, 1995.

Achen, M.G. et al., "Vascular Endothelial Growth Factor D (VEGF-D) is a Ligand for the Tyrosine Kinases VEGF Receptor 2 (Flk1) and VEGF Receptor 3 (Flt4)," *Proceedings of the National Academy of Science, USA*, 95:548-553 (Jan., 1998).

Adams, M.D. et al., "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence," *Nature*, 377(6547 Supple-ment):3-174 (Sep., 1995).

Cohen, T. et al., "VEGF121, A Vascular Endothelial Growth Factor (VEGF) Isoform Lacking Heparin Binding Ability, Requires Cell-Surface Heparan Sulfates for Efficient Binding to the VEGF Receptors of Human Melanoma Cells," *Journal of Biological Chemistry*, 270(19):11322-11326 (May 12, 1995).

Genbank AA151613, "z127h03.s1 Soares pregnant uterus NbHPU *Homo sapiens* cDNA clone 503189 3'," Hillier, L. et al., Dated May 14, 1997.

Genbank AA425486, "zw46b06.r1 Soares total fetus Nb2HF8 9w *Homo sapiens* cDNA clone 773075 5' similar to SW:VEGF_Mouse Q00731 Vascular Endothelial Growth Factor Precursor," Deposited by Hillier, L. et al. Dated Oct. 16, 1997.

Genbank N31713, "yy15b12.s1 *Homo sapiens* cDNA clone 271295 3'," Deposited by Hillier, L. et al. Dated Jan. 10, 1996.

Genbank N31720, "yy15d12.s1 *Homo sapiens* cDNA clone 271319 3'," Deposited by Hillier, L. et al. Dated Jan. 10, 1996.

Genbank AA406492, "zv12g06.r1 Soares NhHMPu S1 *Homo sapiens* cDNA clone 75366 5'," Deposited by Hillier, L. et al. Dated May 17, 1997.

Genbank N50972, "yy94b08.s1 *Homo sapiens* cDNA clone 281175 3'," Deposited by Hillier, L. et al. Dated Feb. 14, 1996.

Genbank AA421713, "zu24b03.s1 Soares NhHMPu S1 *Homo sapiens* cDNA clone 738893 3'," Deposited by Hillier, L. et al. Dated Oct. 16, 1997.

Genbank N94399, "zb76f04.s1 Soares senescent fibroblasts NbHSF *Homo sapiens* cDNA clone 309535 3'," Deposited by Hillier, L. et al. Dated Aug. 20, 1996.

Genbank H05177, "y185b08.r1 *Homo sapiens* cDNA clone 44993 5'," Deposited by Hillier, L. et al. Dated Jun. 21, 1995.

(List continued on next page.)

Primary Examiner—Christine Saoud
(34) Attorney, Agent, or Firm—Marshall, O'Toole, Gerstein, Murray & Borun

(57) ABSTRACT

Provided are ligands for the receptor tyrosine kinase, Flt4. Also provided are cDNAs and vectors encoding the ligand, pharmaceutical compositions and diagnostic reagents.

29 Claims, 16 Drawing Sheets

OTHER PUBLICATIONS

- Genbank AA479987, "zv18h12.s1 Soares NhHMPu S1 *Homo sapiens* cDNA clone 754055 3'," Deposited by Hillier, L. et al. Dated Aug. 8, 1997.
- Genbank H05134, "y185b08.s1 *Homo sapiens* cDNA clone 44993 3'," Deposited by Hillier, L. et al. Dated Jun. 21, 1995.
- Genbank, AA298182 "EST113866 Bone VII *Homo sapiens* cDNA 5' end," Deposited by Adams, M.D. et al. Dated Apr. 18, 1997.
- Genbank AA298283, "EST113896 Bone VII *Homo sapiens* cDNA 5' end similar to vascular endothelial growth factor," Deposited by Adams, M.D. et al. Dated Apr. 18, 1997.
- Genbank T81481, "yd29f07.s1 *Homo sapiens* cDNA clone 109669 3'," Deposited by Hillier, L. et al. Dated Mar. 15, 1995.
- Genbank AA425303, "zw46b06.s1 Soares total fetus Nb2HF8 9w *Homo sapiens* cDNA clone 773075 3', mRNA sequence," Deposited by Hillier, L. et al. Dated Oct. 16, 1997.
- Genbank Z40230, "*H. sapiens* partial cDNA sequence: clone c-1wf11," Deposited by Genexpress. Dated Sep. 21, 1995.
- Genbank Z44272, "*H. sapiens* partial cDNA sequence: clone c-1wf11," Deposited by Genexpress. Dated Sep. 21, 1995.
- Genbank AA478766, "zv18h12.r1 Soares NhHMPu S1 *Homo sapiens* cDNA clone 754055 5'," Deposited by Hillier, L. et al. Dated Aug. 8, 1997.
- Genbank H96876, "yw04b12.s1 Soares melanocyte 2NbHM *Homo sapiens* cDNA clone 251231 3'," Deposited by Hillier, L. et al. Dated Nov. 25, 1996.
- Genbank H96533, "yw04b12.r1 Soares melanocyte 2NbHM *Homo sapiens* cDNA clone 251231 5'," Deposited by Hillier, L. et al. Dated Nov. 25, 1996.
- Genbank T81690, "yd29f07.r1 *Homo sapiens* cDNA clone 109669 5' similar to SP:BAR3_Chite Q03376 Balbiani Ring Protein 3," Deposited by Hillier, L. et al. Dated Mar. 15, 1995.
- Genbank T84377, "yd37h08.r1 *Homo sapiens* cDNA clone 110463 5' similar to SP:BAR3_Chite Q03376 Balbiani Ring Protein 3," Deposited by Hillier, L. et al. Dated Mar. 16, 1995.
- Genbank N42368, "yy15b11.r1 *Homo sapiens* cDNA clone 271293 5'," Deposited by Hillier, L. et al. Dated Jan. 25, 1996.
- Genbank N42374, "yy15d11.r1 *Homo sapiens* cDNA clone 271317 5'," Deposited by Hillier, L. et al. Dated Jan. 25, 1996.
- Genbank H81868, "yv83d09.s1 *Homo sapiens* cDNA clone 249329 3'," Deposited by Hillier, L. et al. Dated Nov. 9, 1995.
- Genbank H81867, "yv83d09.r1 *Homo sapiens* cDNA clone 249329 5'," Deposited by Hillier, L. et al. Dated Nov. 9, 1995.
- Genbank AA149461, "z127h03.r1 Soares pregnant uterus NhHMPu *Homo sapiens* cDNA clone 503189 5' similar to SW:BAR3_Chite Q03376 Balbiani Ring Protein 3 Precursor," Deposited by Hillier, L. et al. Dated May 14, 1997.
- Genbank R77495, "y179e04.s1 *Homo sapiens* cDNA clone 145470 3'," Deposited by Hillier, L. et al. Dated Jun. 7, 1995.
- Genbank H07899, "y186g06.s1 *Homo sapiens* cDNA clone 45138 3'," Deposited by Hillier, L. et al. Dated Jun. 23, 1995.
- Genbank T89295, "yd37h08.s1 *Homo sapiens* cDNA clone 110463 3'," Deposited by Hillier, L. et al. Dated Mar. 20, 1995.
- Genbank C21512, "HUMGS0010510, Human Gene Signature, 3'-directed cDNA sequence," Deposited by Okubo, K. Dated Oct. 1, 1996.
- Genbank N82975, "TgESTzy53h10.r1 TgRH Tachyzoite cDNA *Toxoplasma gondii* cDNA clone tgy53h10.r1 5'," Deposited by Hehl, A. et al. Dated Sep. 10, 1997.
- Genbank AA285997, "vb88h06.r1 Soares mouse 3NbMS *Mus musculus* cDNA clone 764123 5'," Deposited by Marra, M. et al. Dated Apr. 9, 1997.
- Genbank AA549856, "0929m3 gmbPFB3.1, G. Roman-Reddy *Plasmodium falciparum* genomic clone 0929m," Deposited by Dame, J.B. et al. Dated Aug. 11, 1997.
- Jeltsch, M. et al., "Hyperplasia of Lymphatic Vessels in VEGF-C Transgenic Mice," *Science*, 276:1423-1425 (May, 1997).
- Joukov, V. et al., "Proteolytic Processing Regulates Receptor Specificity and Activity of VEGF-C," *EMBO Journal*, 16(13):3898-3911 (Jun., 1997).
- Joukov, V. et al., "A Recombinant Mutant Vascular Endothelial Growth Factor-C that has Lost Vascular Endothelial Growth Factor Receptor-2 Binding, Activation, and Vascular Permeability Activities," *Journal of Biological Chemistry*, 273(12):6599-6602 (Mar. 20, 1998).
- Lee, J. et al., "Vascular Endothelial Growth Factor Related Protein (vrp): A Ligand and Specific Activator of the Tyrosine Kinase Receptor Flt4," EMBL Sequence Data Library, XP002066361, accession No. U4142, Dated Jan. 10, 1996.
- Hillier et al., "The WashU-Merck EST Project," EMBL Database entry H5991157, accession No. H07991, Jul. 2, 1995.
- Alitalo et al., "Vascular Endothelial Growth Factors and Receptors Involved in Angiogenesis," *The 9th International Conference of the International Society of Differentiation (ISD), Development Cell Differentiation and Cancer*, Pisa (Italy), Sep. 28-Oct. 2, 1996, p. 66 (Abstract S22).
- Alitalo et al., "Vascular Endothelial Growth Factors B and C Receptors Involved in Angiogenesis," *German-American Academic Council Foundation (GAACF) Stiftung Deutsch-Amerikanisches Akademisches Konzil (DAAK), 2nd Symposium on Current Problems in Molecular Medicine: The Role of Cytokines in Human Disease*, Nov. 17-20, 1996, Ringberg Castle, Germany, p. 1 (Abstract).
- Andersson et al., "Assignment of Interchain Disulfide Bonds in platelet-Derived Growth Factor (PDGF) and Evidence for Agonist Activity of Monomeric PDGF," *J. Biol. Chem.*, 267(16):11260-11266 (Jun. 5, 1992).
- Aprelikova et al., "FLT4, A Novel Class III Receptor Tyrosine Kinase in Chromosome 5q33-qter," *Cancer Research*, 52:746-748 (Feb. 1, 1992).
- Bastien et al., "The FGF Family of Growth Factors and Oncogenes," *Adv. Cancer Res.*, 59:145-165 (1992).
- Berse et al., "Vascular Permeability Factor (Vascular Endothelial Growth Factor) Gene is Expressed Differentially in Normal Tissues, Macrophages, and Tumors," *Mol. Biol. Cell.*, 3:211-220 (Feb., 1992).
- Betscholtz et al., "cDNA Sequence and Chromosomal Localization of Human Platelet-Derived Growth Factor A-Chain and Its Expression in Tumor Cell Lines," *Nature*, 320:695-699 (Apr., 1986).

- Borg et al., "Biochemical Characterization of Two Isoforms of FLT4, a VEGF Receptor-Related Tyrosine Kinase," *Oncogene*, 10:973-84 (1995).
- Cao et al., "Heterodimers of Placenta Growth Factor/Vascular Endothelial Growth Factor," *J. Biol. Chem.*, 271(6):3154-3162 (Feb. 9, 1996).
- Cheng and Flanagan, "Identification and Cloning of ELF-1, A Developmentally Expressed Ligand for the Mek4 and Sek Receptor Tyrosine Kinases," *Cell*, 79:157-168 (Oct. 7, 1994).
- Claesson-Welsh et al., "Identification and Structural Analysis of the A Type Receptor for Platelet-derived Growth Factor," *J. Biol. Chem.*, 264(3):1742-1747 (Jan. 25, 1989).
- Coffin et al., "Angioblast Differentiation and Morphogenesis of the Vascular Endothelium in the Mouse Embryo," *Devel. Biol.*, 148:51-62 (1991).
- Curran and Franza, "Fos and Jun: The AP-1 Connection," *Cell*, 55:395-397 (Nov. 4, 1988).
- De Vries et al., "The fms-Like Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor," *Science*, 255:989-991 (Feb. 21, 1992).
- DiSalvo et al., "Purification and Characterization of a Naturally Occurring Vascular Endothelial Growth Factor: Placenta Growth Factor Heterodimer," *J. Biol. Chem.*, 270(13):7717-7723 (Mar. 31, 1995).
- Dumont et al., "Dominant-negative and Targeted Null Mutations in the Endothelial Receptor Tyrosine Kinase, tek, Reveal a Critical Role in Vasculogenesis of the Embryo," *Genes Dev.*, 8:1897-1909 (1994).
- Dumont et al., "Vascularization of the Mouse Embryo: A Study of flk-1, tek, tie and Vascular Endothelial Growth Factor Expression During Development," *Development Dynamics*, 203:80-92 (1995).
- Dvorak et al., "Review: Vascular Permeability Factor/Vascular Endothelial Growth Factor, Microvascular Hyperpermeability, and Angiogenesis," *Amer. J. Path.*, 146:1029-1039 (1995).
- Eichmann et al., "Two Molecules Related to the VEGF Receptor are Expressed in Early Endothelial Cells During Avian Embryonic Development," *Mech. Dev.*, 42:33-48 (1993).
- Ferrara et al., "Molecular and Biological Properties of the Vascular Endothelial Growth Factor Family of Proteins," *Endocrine Rev.*, 13(1):18-32 (1992).
- Finnerty et al., "Molecular Cloning of Murine FLT and FLT4," *Oncogene*, 8(11):2293-2298 (1993).
- Flamme et al., "Vascular Endothelial Growth Factor (VEGF) and VEGF-Receptor 2 (flk-1) are Expressed During Vasculogenesis and Vascular Differentiation in the Quail Embryo," *Devel. Biol.*, 169:699-712 (1995).
- Flanagan and Leder, "The kit Ligand: A Cell Surface Molecule Altered in Steel Mutant Fibroblasts," *Cell*, 63:185-194 (Oct. 5, 1990).
- Folkman, "Angiogenesis in Cancer, Vascular, Rheumatoid and Other Disease," *Nature Med.*, 1(1):27-31 (1995).
- Folkman et al., "Long-term Culture of Capillary Endothelial Cells," *Proc. Nat'l. Acad. Sci., USA*, 76(10):5217-5221 (Oct. 1979).
- Fong et al., "Role of the Flt-1 Receptor Tyrosine Kinase in Regulating the Assembly of Vascular Endothelium," *Nature*, 376:66-70 (Jul. 6, 1995).
- Friesel et al., "Molecular Mechanisms of Angiogenesis: Fibroblast Growth Factor Signal Transduction," *FASEB J.*, 9:919-25 (1995).
- Genbank S66407, "FLT4 Receptor Tyrosine Kinase Isoform FLT4 Long (3' Region, Alternatively Spliced) (Human, mRNA Partial, 216 nt)," Deposited by Pajusola et al., Dated Dec. 17, 1993.
- Genbank U48800, "Mus Musculus Vascular Endothelial Growth Factor B Precursor (VEGF-B) mRNA, Complete Cds," Deposited by Olofsson et al., Dated Aug. 19, 1996.
- Genbank X15997, "Human Vascular Permeability Factor mRNA, Complete Cds," Deposited by Keck et al., Dated Jun. 15, 1990.
- Genbank X60280, "Vector Plasmid pLTRpoly DNA," Deposited by Mackelae, T.P., Dated Jul. 16, 1996.
- Genbank X68203, "H. sapiens mRNA for FLT4, Class III Receptor Tyrosine Kinase," Deposited by Aprelikova, O., Dated Nov. 30, 1993.
- Genbank X94216, "Homo sapiens mRNA for VEGF-C protein," Deposited by Joukov et al., Dated Feb. 6, 1996.
- Harlow et al., *Antibodies, a Laboratory Manual*, Cold Spring Harbor Laboratory Press, pp. 72-137, 141-157, 287 & 321-358 (1988).
- Heldin et al., "Structure of Platelet-Derived Growth Factor: Implications Functional Properties," *Growth Factors*, 8:245-252 (1993).
- Joukov et al., "A Novel Vascular Endothelial Growth Factor, VEGF-C, Is a Ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) Receptor Tyrosine Kinases," *EMBO J.*, 15(2):290-298 (1996).
- Kaipainen et al., "Expression of the FMS-Like Tyrosine Kinase 4 Gene Becomes Restricted to Lymphatic Endothelium During Development," *Proc. Nat'l Acad. Sci., USA*, 92:3566-3570 (Apr., 1995).
- Kaipainen et al., "The Related FLT4, FLT1 and KDR Receptor Tyrosine Kinases Show Distinct Expression Patterns in Human Fetal Endothelial Cells," *J. Exp. Med.*, 178:2077-2088 (Dec., 1993).
- Kaipainen et al., "Enhanced Expression of the Tie Receptor Tyrosine Kinase Messenger RNA in the Vascular Endothelium of Metastatic Melanomas," *Cancer Res.*, 54:6571-6577 (Dec. 15, 1994).
- Kukkk et al., "VEGF-C Receptor Binding and Pattern of Expression with VEGFR-3 Suggests a Role in Lymphatic Vascular Development," *Development*, 122:3829-3837 (1996).
- Lee et al., "Vascular Endothelial Growth Factor-Related Protein: A Ligand and Specific Activator of the Tyrosine Kinase Receptor Flt4," *Proc. Nat'l Acad. Sci., USA*, 93:1988-1992 (Mar., 1996).
- Leung et al., "Vascular Endothelial Growth Factor Is a Secreted Angiogenic Mitogen," *Science*, 246:1306-1309 (Dec. 8, 1989).
- Levy et al., "Post-transcriptional Regulation of Vascular Endothelial Growth Factor by Hypoxia," *J. Biol. Chem.*, 271(5):2746-2753 (Feb. 2, 1996).
- Levy et al., "Transcriptional Regulation of the Rat Vascular Endothelial Growth Factor Gene by Hypoxia," *J. Biol. Chem.*, 270(22):13333-13340 (Jun. 2, 1995).
- Lyman et al., "Molecular Cloning of a Ligand for the flk/flk-2 Tyrosine Kinase Receptor: A Proliferative Factor for Primitive Hematopoietic Cells," *Cell*, 75:1157-1167 (Dec. 17, 1993).
- Maglione et al., "Isolation of a Human Placenta cDNA Coding for a Protein Related to the Vascular Permeability Factor," *Proc. Nat'l Acad. Sci., USA*, 88:9267-9271 (Oct., 1991).

- Maglione et al., "Two Alternative mRNAs Coding for the Angiogenic Factor, Placenta Growth Factor (PIGF) are Transcribed from a Single Gene of Chromosome 14," *Oncogene*, 8:925-931 (1993).
- Matthews et al., "A Receptor Tyrosine Kinase cDNA Isolated from a Population of Enriched Primitive Hematopoietic Cells and Exhibiting Close Genetic Linkage to c-kit," *Proc. Nat'l Acad. Sci., USA*, 88:9026-9030 (Oct., 1991).
- Metzelaar et al., "CD63 Antigen," *J. of Biol. Chem.*, 266(5):3239-3245 (Feb. 15, 1991).
- Millauer et al., "Glioblastoma Growth Inhibited in vivo by a Dominant-Negative Flk-1 Mutant," *Nature*, 367:576-579 (Feb. 10, 1994).
- Millauer et al., "High Affinity VEGF Binding and Developmental Expression Suggest Flk-1 as a Major Regulator of Vasculogenesis and Angiogenesis," *Cell*, 72:835-846 (Mar. 26, 1993).
- Mitchell et al., "Transcription Factor AP-2 is Expressed in Neural Crest Cell Lineages During Mouse Embryogenesis," *Genes and Dev.*, 5:105-119 (1991).
- Morgenstern et al., "Advanced Mammalian Gene Transfer: High Titer Retroviral Vectors With Multiple Drug Selection Markers and a Complementary Helper-Free Packaging Cell Line," *Nucl. Acids Res.*, 18(12):3587-3595 (1990).
- Mount, S.M., "A Catalogue of Splice Junction Sequences," *Nucl. Acids Res.*, 10(2):459-472 (1982).
- Mustonen et al., "Endothelial Receptor Tyrosine Kinases Involved in Angiogenesis," *J. Cell Biol.*, 129:895-898 (May, 1995).
- Nelson and Sun, "The 50- and 58-kdalton Keratin Classes as Molecular Markers for Stratified Squamous Epithelia: Cell Culture Studies," *J. Cell Biol.*, 97:244-251 (Jul., 1983).
- Neufeld et al., "Vascular Endothelial Growth Factor and Its Receptors," *Prog. Growth Fact. Res.*, 5:89-97 (1994).
- Oefner et al., "Crystal Structure of Human Platelet-derived Growth Factor BB," *EMBO J.*, 11(11):3921-3926 (1992).
- Oelrichs et al., "NYK/FLK-1: A Putative Receptor Tyrosine Kinase Isolated from E10 Embryonic Neuroepithelium is Expressed in Endothelial Cells of the Developing Embryo," *Oncogene*, 8:11-18 (1993).
- Olofsson et al., "Vascular Endothelial Growth Factor B, A Novel Growth Factor for Endothelial Cells," *Proc. Nat'l Acad. Sci., USA*, 93:2576-2581 (Mar., 1996).
- Paavonen et al., "Chromosomal Localization and Regulation of Human Vascular Endothelial Growth Factors B and C (VEGF-B and VEGF-C)," *IX International Vascular Biology Meeting*, Seattle, Washington, Sep. 4-8, 1996, p. 76 (Abstract 299).
- Paavonen et al., "Novel Human Vascular Endothelial Growth Factor Genes VEGF-B and VEGF-C Localize to Chromosomes 11q13 and 4q34, Respectively," *Circulation*, 93(6):1079-1082 (Mar. 15, 1996).
- Park et al., "Placenta Growth Factor, Potentiation of Vascular Endothelial Growth Factor Bioactivity In vitro and In vivo, and High Affinity Binding to Flt-1 but not to Flk-1/KDR," *J. Biol. Chem.*, 269(41):25646-25654 (Oct. 14, 1994).
- Partanen et al., "A Novel Endothelial Cell Surface Receptor Tyrosine Kinase with Extracellular Epidermal Growth Factor Homology Domains," *Mol. & Cell. Biol.*, 12(4):1698-1707 (Apr., 1992).
- Partanen et al., "Putative Tyrosine Kinase Expressed in K-562 Human Leukemia Cells," *Proc. Nat'l Acad. Sci., USA*, 87:8913-8917 (Nov., 1990).
- Paulsson et al., "The Balbiani Ring 3 Gene in *Chironomus tentans* has a Diverged Repetitive Structure Split by Many Introns," *J. Mol. Biol.*, 211:331-349 (1990).
- Pear et al., "Production of High-titer Helper-free Retroviruses by Transient Transfection," *Proc. Nat'l Acad. Sci., USA*, 90:8392-8396 (Sep., 1993).
- Pertovaara et al., "Vascular Endothelial Growth Factor Is Induced in Response to Transforming Growth Factor- β in Fibroblastic and Epithelial Cells," *J. Biol. Chem.*, 269(9):6271-6274 (Mar. 4, 1994).
- Peters et al., "Vascular Endothelial Growth Factor Receptor Expression during Embryogenesis and Tissue Repair Suggests a Role in Endothelial Differentiation and Blood Vessel Growth," *Proc. Nat'l Acad. Sci., USA*, 90:8915-8918 (Oct., 1993).
- Pötgens et al., "Covalent Dimerization of Vascular Permeability Factor/Vascular Endothelial Growth Factor Is Essential for Its Biological Activity," *J. Biol. Chem.*, 269(52):32879-32885 (Dec. 30, 1994).
- Puri et al., "The Receptor Tyrosine Kinase TIE is Required for Integrity and Survival of Vascular Endothelial Cells," *EMBO J.*, 14:5884-5891 (1995).
- Quinn et al., "Fetal Liver Kinase 1 is a Receptor for Vascular Endothelial Growth Factor and is Selectively Expressed in Vascular Endothelium," *Proc. Nat'l Acad. Sci., USA*, 90:7533-7537 (Aug., 1993).
- Risau et al., "Platelet-Derived Growth Factor is Angiogenic In Vivo," *Growth Factors*, 7:261-266 (1992).
- Risau, W., "Differentiation of Endothelium," *FASEB J.*, 9:926-933 (1995).
- Sabin, F.R., "The Lymphatic System in Human Embryos, With A Consideration of the Morphology of the System as a Whole," *Am. J. Anat.*, 9(1):43-91 (1909).
- Sambrook et al., *Molecular Cloning: a Laboratory Manual*, Second Edition, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory (1989), pp. 2.60-2.79, 4.21-4.32, 7.3-7.36, and 9.47-9.51.
- Sato et al., "Distinct Roles of the Receptor Tyrosine Kinases Tie-1 and Tie-2 in Blood Vessel Formation," *Nature*, 376:70-74 (Jul. 6, 1995).
- Schneider et al., "A One-step Purification of Membrane Proteins Using a High Efficiency Immunomatrix," *J. Biol. Chem.*, 257(18):10766-10769 (Sep. 25, 1982).
- Seetharam et al., "A Unique Signal Transduction from FLT Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor VEGF," *Oncogene*, 10:135-147 (1995).
- Senger et al., "Tumor Cells Secrete a Vascular Permeability Factor That Promotes Accumulation of Ascites Fluid," *Science*, 219:983-985 (Feb. 25, 1983).
- Shalaby et al., "Failure of Blood-Island Formation and Vasculogenesis in Flk-1-deficient Mice," *Nature*, 376:62-66 (Jul. 6, 1995).
- Shibuya et al., "Nucleotide Sequence and Expression of a Novel Human Receptor-Type Tyrosine Kinase Gene (flt) Closely Related to the fms Family," *Oncogene*, 5:519-524 (1990).
- Shibuya, M., "Role of VEGF-FLT Receptor System in Normal and Tumor Angiogenesis," *Adv. Cancer Res.*, 67:281-316 (1995).
- Shweiki et al., "Patterns of Expression of Vascular Endothelial Growth Factor (VEGF) and VEGF Receptors in Mice Suggest a Role in Hormonally Regulated Angiogenesis," *J. Clin. Invest.*, 91:2235-2243 (May, 1993).

- Southern and Berg, "Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter," *J. Mol. Appl. Genet.*, 1:327-341 (1982).
- Terman et al., "Identification of New Endothelial Cell Growth Factor Receptor Tyrosine Kinase," *Oncogene*, 6:1677-1683 (1991).
- Terman et al., "Identification of the KDR Tyrosine Kinase as a Receptor for Vascular Endothelial Cell Growth Factor," *Biochem. Biophys. Res. Commun.*, 187:1579-1586 (Sep. 30, 1992).
- Terman et al., "VEGF Receptor Subtypes KDR and FLT1 Show Different Sensitivities to Heparin and Placenta Growth Factor," *Growth Factors*, 11(3):187-195 (1994).
- Tischer et al., "The Human Gene for Vascular Endothelial Growth Factor. Multiple Protein Forms are Encoded Through Alternative Exon Splicing," *J. Biol. Chem.*, 266(18):11947-11954 (Jun. 25, 1991).
- Vassár et al., "Tissue-specific and Differentiation-specific Expression of a Human K14 Keratin Gene in Transgenic Mice," *Proc. Nat'l Acad. Sci., USA*, 86:1563-1567 (Mar., 1989).
- Vassár et al., "Transgenic Mice Provide New Insights Into the Role of TGF- α During Epidermal Development and Differentiation," *Genes & Dev.*, 5:714-727 (1991).
- Väström et al., "Expression of the Mad Gene During Cell Differentiation In Vivo and Its Inhibition of Cell Growth In Vitro," *J. Cell. Biol.*, 128(6):1197-1208 (Mar., 1995).
- von Heijne, G., "A New Method for Predicting Signal Sequence Cleavage Sites," *Nucleic Acids Res.*, 14(11):4683-4690 (1986).
- Waltenberger et al., "Different Signal Transduction Properties of KDR and Flt1, Two Receptors for Vascular Endothelial Growth Factor," *J. Biol. Chem.*, 269(43):26988-26995 (Oct. 28, 1994).
- Wanaka et al., "Expression of FGF Receptor Gene in Rat Development," *Development*, 111:455-468 (1991).
- Wen et al., "Neu Differentiation Factor: A Transmembrane Glycoprotein Containing an EGF Domain and an Immunoglobulin Homology Unit," *Cell* 69:559-572 (May 1, 1992).
- Yamane et al., "A New Communication System Between Hepatocytes and Sinusoidal Endothelial Cells in Liver Through Vascular Endothelial Growth Factor and Flt Tyrosine Kinase Receptor Family (Flt-1 and KDR/Flk-1)," *Oncogene*, 9:2683-2690 (1994).
- Ausprunk, et al., "Migration and Proliferation of Endothelial Cells in Preformed and Newly Formed Blood Vessels during Tumor Angiogenesis," *Microvascular Research*, 14:53-65 (1977).
- Breier, et al., "Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation," *Development*, 114:521-532 (1992).
- Dignam, et al., "Balbiani ring 3 in *Chironomus tentans* encodes a 185-kDa secretory protein which is synthesized throughout the fourth larval instar," *Gene*, 88:133-140 (1990).
- Don, et al., "'Touchdown' PCR to circumvent spurious priming during gene amplification," *Nucleic Acids Research*, 19(14):4008 (1991).
- Folkman, et al., "Angiogenesis," *The Journal of Biological Chemistry*, 267(16):10931-10934 (1992).
- Kozak, "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs," *Nucleic Acids Research*, 15(20):8125-8148 (1987).
- Mäkelä, et al., "Plasmid pLTRpoly: A Versatile High-Efficiency Mammalian Expression Vector," *Gene*, 118:293-294 (1992).
- Pajusola, et al., "FLT4 Receptor Tyrosine Kinase Contains Seven Immunoglobulin-like Loops and Is Expressed in Multiple Human Tissues and Cell Lines," *Cancer Research*, 52:5738-5743 (1992).
- Pajusola, et al., "Two human FLT4 receptor tyrosine kinase isoforms with distinct carboxy terminal tails are produced by alternative processing of primary transcripts," *Oncogene*, 8:2931-2937 (1993).
- Risau, et al., "Changes in the Vascular Extracellular Matrix during Embryonic Vasculogenesis and Angiogenesis," *Developmental Biology*, 125:441-450 (1988).
- Saksela, et al., "Cell-Associated Plasminogen Activation: Regulation and Physiological Functions," *Annu. Rev. Cell. Biol.*, 4:93-126 (1988).
- Tessier, et al., "Enhanced secretion from insect cells of a foreign protein fused to the honeybee melittin signal peptide," *Gene*, 98:177-183 (1991).
- van der Geer, et al., "Receptor Protein-Tyrosine Kinases and Their Signal Transduction Pathways," *Annu. Rev. Cell. Biol.*, 10:251-337 (1994).

* cited by examiner

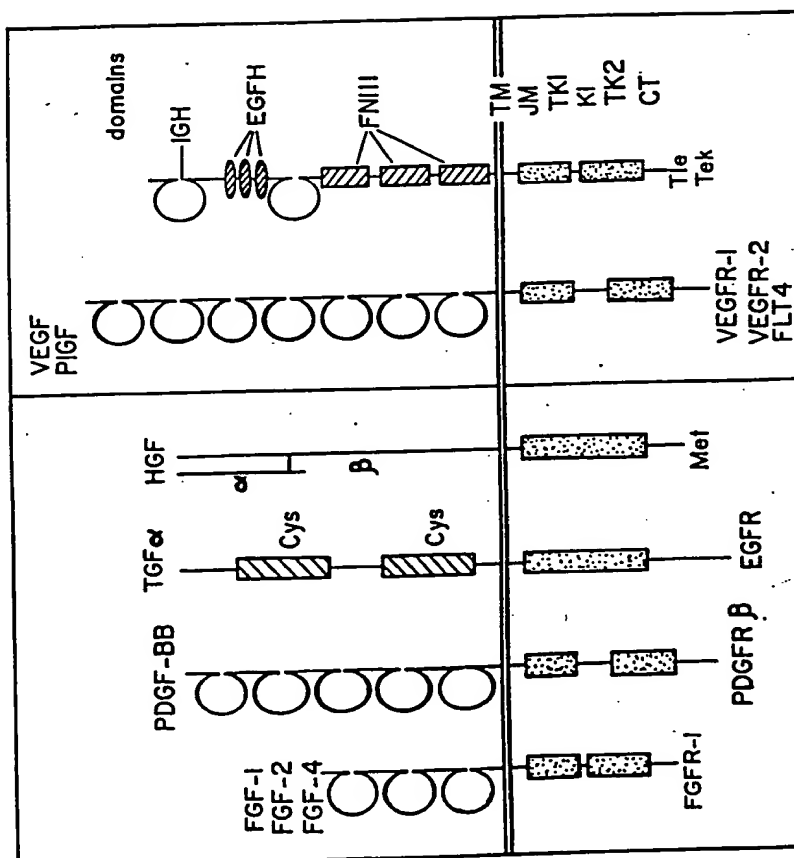


FIGURE 1

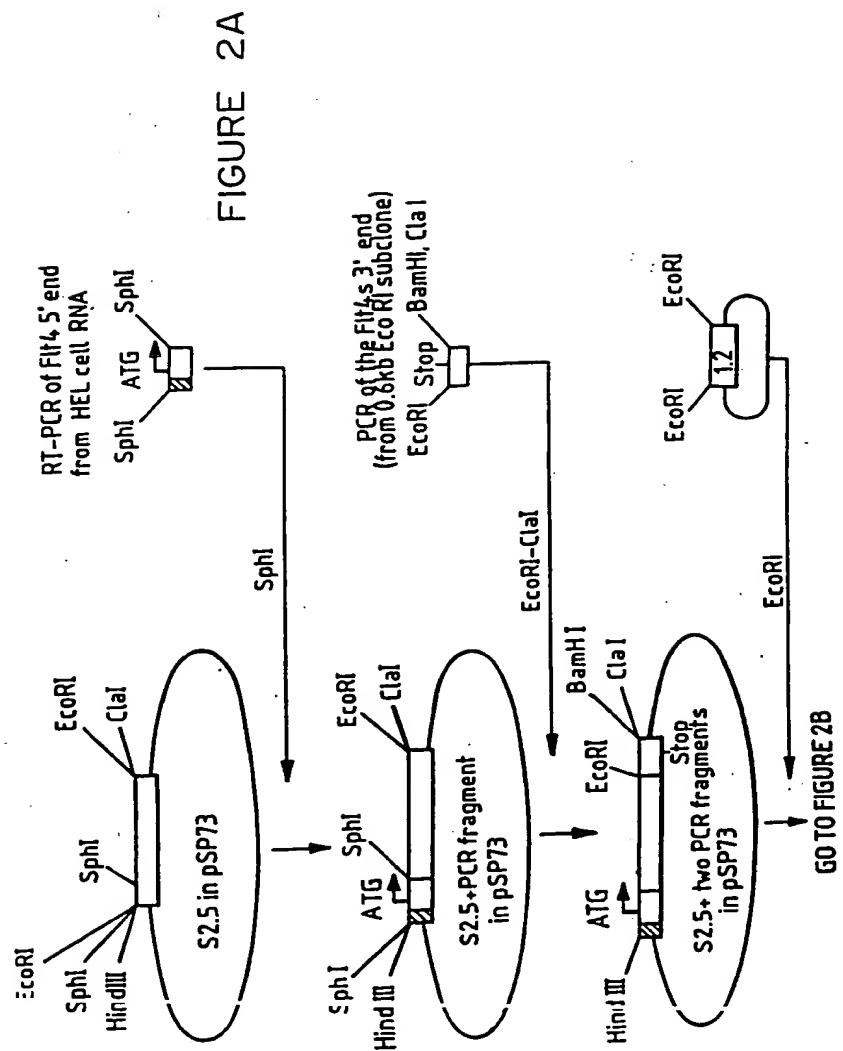
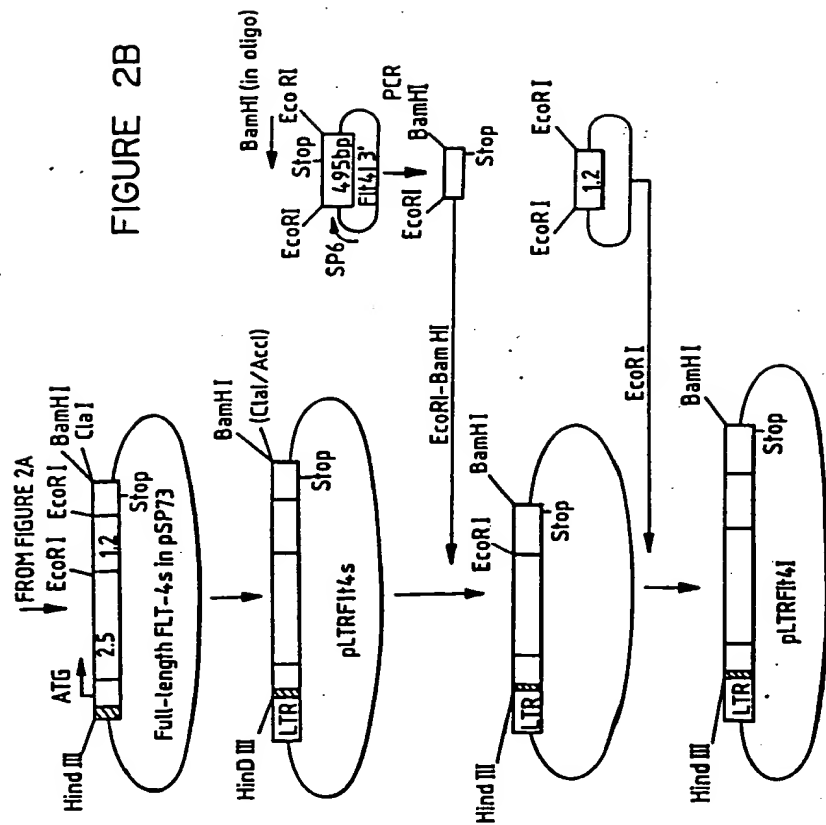


FIGURE 2B



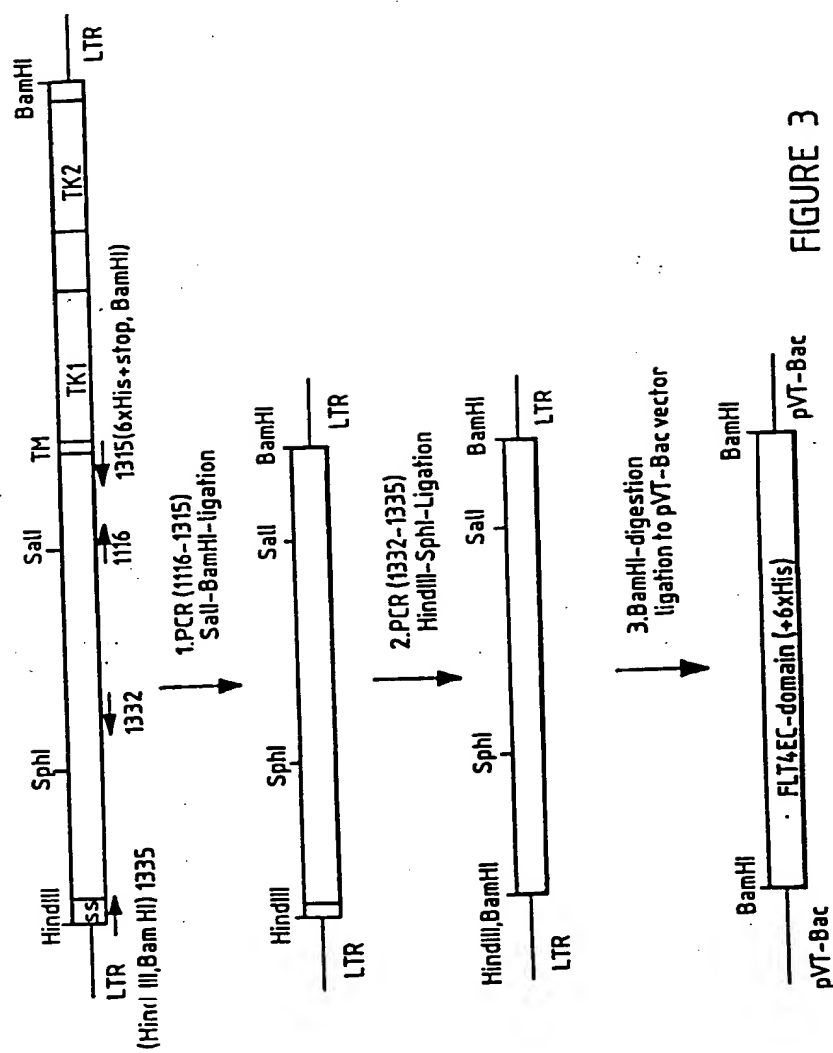


FIGURE 3

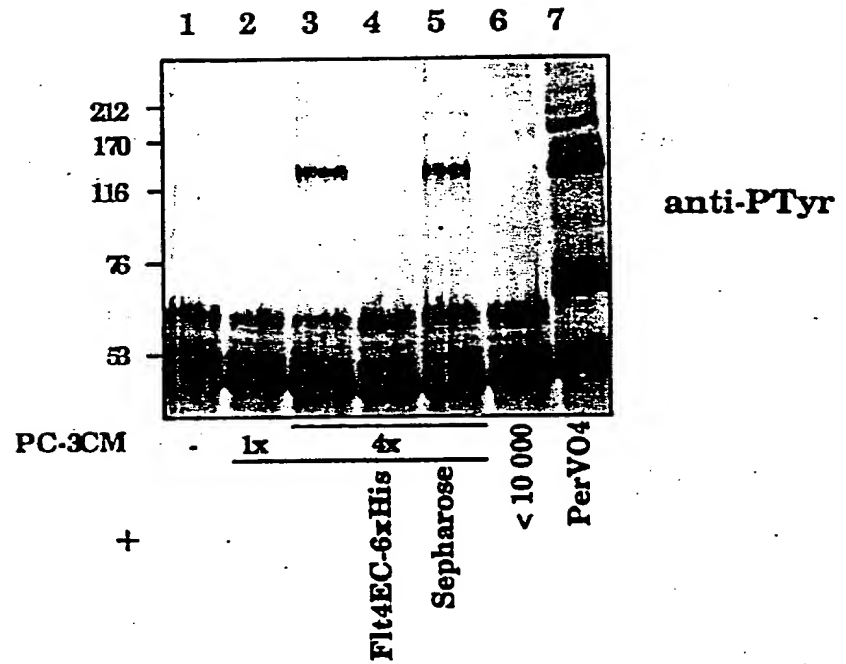


FIGURE 4

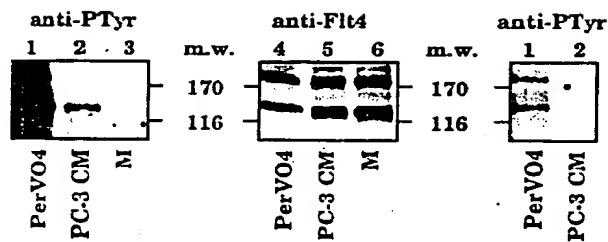


FIGURE 5A

FIGURE 5B

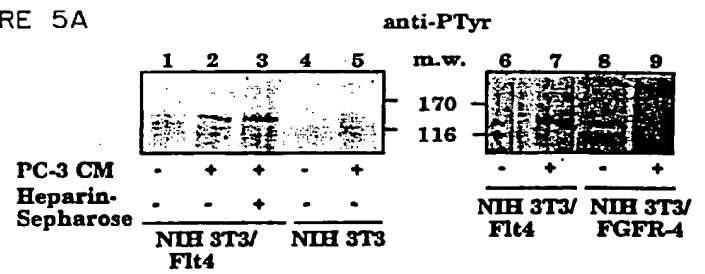


FIGURE 5C

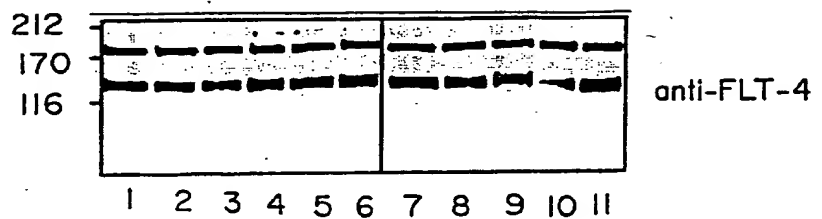


FIGURE 6A

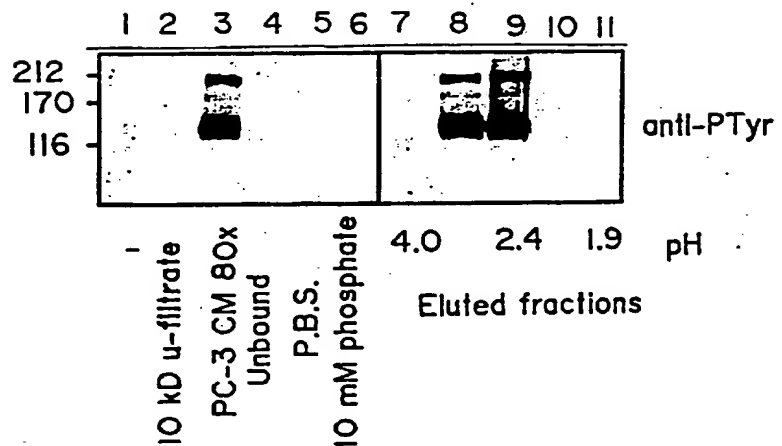


FIGURE 6B

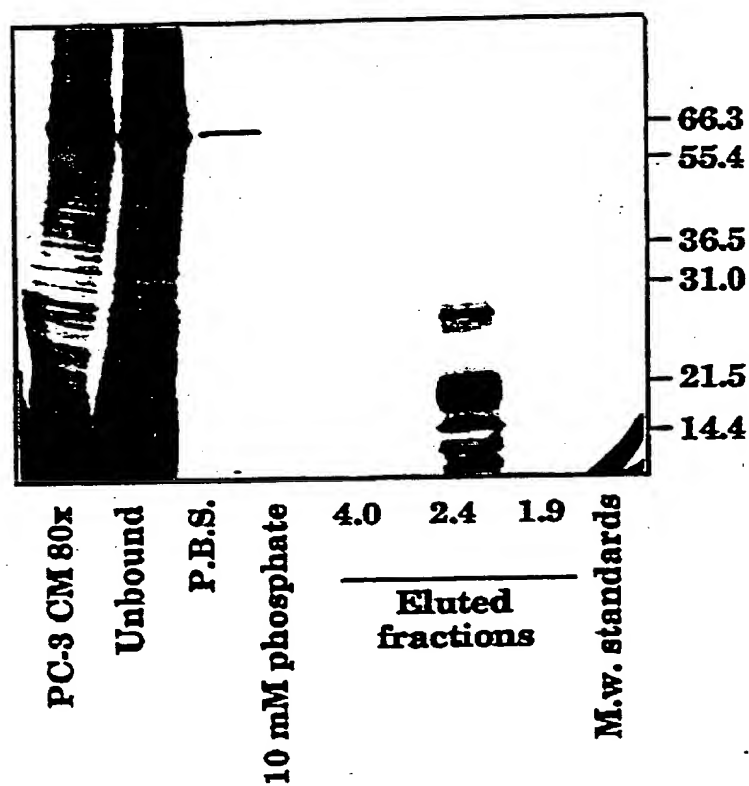


FIGURE 7

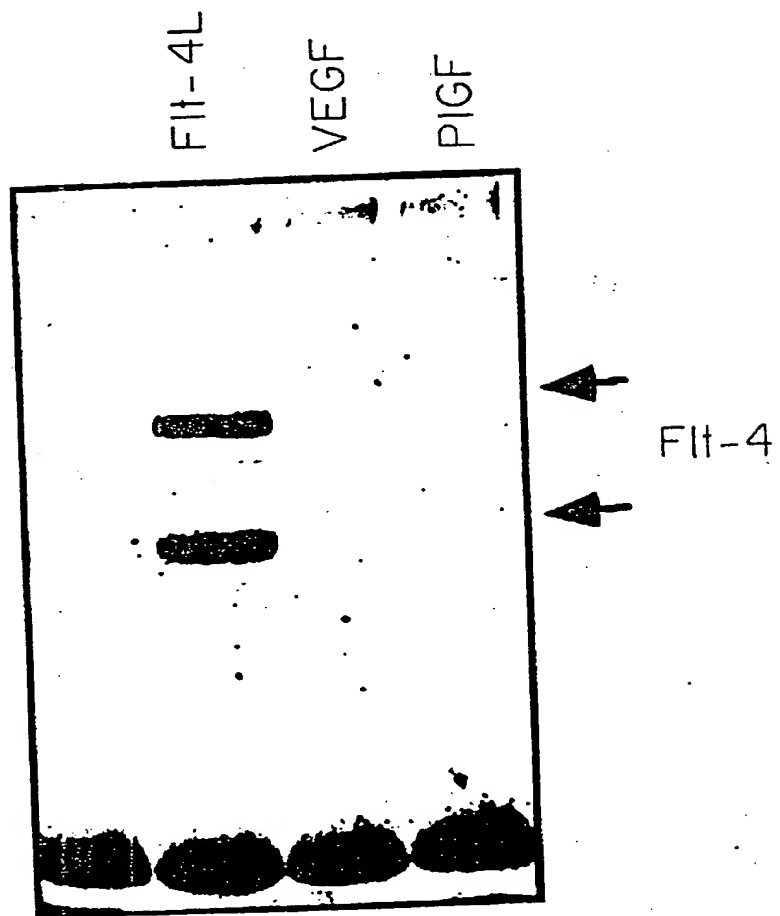


FIGURE 8

MetThrValLeuTyrProGluTyr
GAC(CAGTTACGGTCTGTGTCCAGTGTAGATGAACCTCATGTACTGTACTCTACCCAGAAATAT 50
10 30
TrpLysMetTyrLysCysGlnLeuArgLysGlyGlyTyrGlnHisAsnArgGluGlnAla
TGAAAAATGTACAAGTGTGAGCTAAGGAAAGGAGGCTGGCAACATAACAGAGAACAGGCC 110
70 90
AsnLeuAsnSerArgThrGluGluThrIleLysPheAlaAlaHisTyrAsnThrGlu
AACCTCAACTCAAGGACAGAGAGACTATAAAATTGTCTGCAGCACATTATATAATACAGAG 170
130 150
IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys
ATCTTGAAAAAGTATTGATTAATGAGTGGAGAAAGACTCAATGCATGCCACGGGAGGTGTGT 230
190 210
IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProProCysVal
ATPAGATGTGGGGAAGGAGTTTGGAGTCGCGACAAACACCTTCTTTAAACCTCCATGTGTG 290
250 270
SerValTyrArgCysGlyCysCysAsnSerGluGlyLeuGlnCysMetAsnThrSer
TCCGTCTACAGATGTGGGGGTTGCTGCAATAGTGAGGGGCTGCAGTGCATGAACACCAGC 350
310 330

FIGURE 9A

ThrSerTyrLeuSerLysThrLeuPheGluIleThrValProLeuSerGlnGlyProLys
ACGAGCTACCTCAGCAAGACGTTATTGAAATTACAGTGCCTCTCTCTCAAGGCCCCCAA
370 390 410

ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal
CCAGTAACAATCAGTTTTTGCCAATCACACTTCCTGCCGATGCATGTCTAAACTGGATGTT
430 450 470

TyrArgGlnValHisSerIleIleArgArgSerLeuProAlaThrLeuProGlnCysGln
TACAGACAAGTTCATTCCATTATTAGACGTTCCCTGCCAGCAACACTACCACAGTGTCTCAG
490 510 530

AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu
GCAGCGAACAAGACCTGCCCCACCAATTACATGTGGAATAATCACATCTGCAGATGCCTG
550 570 590

AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspSerThrAspGlyPheHis
GCTCAGGAAGATTTTATGTTTTCTCGGATGCTGGAGATGACTCAACAGATGGATTCCAT
610 630 650

AspIleCysGlyProAsnLysGluLeuAspGluGluThrCysGlnCysValCysArgAla
GACATCTGTGGACCAACAAGGAGCTGGATGAAGAGACCTGTCTGTCTGCAGAGCG
670 690 710

FIGURE 9B

GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys
GGGCTTCGGCCTGCCAGCTGTGGACCCCAAGAACTAGACAGAACTCATGCCAGTGT
730 750 770

ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAsn
GTCTGTAAAAACAACTCTTCCCCAGCCAATGTGGGCCCAACCGAGAAATTTGATGAAAC
790 810 830

ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys
ACATGCCAGTGTGTATGTATAAAGAACCTGCCCCAGAAATCAACCCCTAAATCCTGGAAAA
850 870 890

CysAlaCysGluCysThrGluSerProGlnLysCysLeuLeuLysGlyLysLysPheHis
TGTGCCTGTGAATGTACAGAAAGTCCACAGAAATGCTTGTAAAGGAAAGAGTTCCAC
910 930 950

HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro
CACCAAAACATGCAGCTGTACAGACGGCCATGTACGAACCGCCAGAGGCTTGTGAGCCA
970 990 1010

GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln
GGATTTTCATATAGTGAAGAAGTGTGCTGTGTGTCCTTCATATTGGAAAGACCCACA
1030 1050 1070

MetSerEnd
ATGAGCTAAGATTGTACTGTTTCCAGTTTCATCGATTTTCTATTATGGAAAACTGTGTTG
1090 1110 1130

FIGURE 9C

1 50
PDGF-A .MRTWACLLL LCGYLALHAL AEEAETPREL IERLARSQIH SIRDQLRLLE
PDGF-B MNRCHA.LFL SLCCYLRLVS AEGDPIPEEL YEMLSHDHSIR SFDDLQRLHL
PLGFMP VMRLFPCFLQ LLAGLAL...
VEGFMNFLLSWVH WSLALLLYLH
FLT4-LMTVLYPEYWK MYKQLRKGG
51 100
PDGF-A IDSVGAEDAL ETSIRAHGSH AINHVPKRP VPTRKRRI.EEAIP
PDGF-B GDP.GEEDGA ELDLNMTRSH SGGELES... .LARGRRSLG SLTIAEPAMI
PLGF PAVPQQWAL SA..... GNGSSEVEV P.FQEVWG..R
VEGF HAKWSQAAPM AE..... GGGQNHHEV K.FMDVYQ..R
FLT4-L WQHNREQANL NSRTEETIKF AAHYNTEIL KSIDNEW..K
101 150
PDGF-A AVCKTRTVIY EIPRSQVDPT SANFLIMPPC VEVKRGCGCC NTSSVKCQPS
PDGF-B AECKTRTEVF EISRLIDRT NANFLVMPPC VEVQCSGCC NNRNVQCRPT
PLGF SYCRALERIV DVVSEY..PS EVEHMFSPSC VSLIRCTGCC GDENLHCVPV
VEGF SYCHPIETLV DIFQY..PD EIEYIFKPC VPLMRCGCC NDEGLECVPT
FLT4-L TQMPREVC I DVGKEF..GV ATNTFFKPC VSVYRQSGCC NSEGLQCMNT
151 200
PDGF-A RVHRSVKVA KVEYVRKKPK LKEVQRLEE FLEQACI.... AT.....
PDGF-B QVQLRPVQVR KIEIVRKKPI FKKATVTLED HLAQCC.... ETVAAARPVT
PLGF ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRCECPRLR EKKPERC...
VEGF EESNITMQIM RIKPH..QQQ .HIGEMSFLQ HNKCECPKPK DRARQENP...
FLT4-L STSYLSKTLF EITVPLSQGP .KPVITISFAN HTSQRQMSKL DVYRQVHSII

FIGURE 10A

201 PDGF-A ..SNLNPDHR EEETDVR... 250
PDGF-B RSPGSGSEQR AKTPQTRVTI RTVRVRPPK GKHRKPKHTH DKTALKETLG
PLGF GDAVPRR...
VEGF CGPCSERRKH LFVQDPQTCK CSCKNTDSRC KARQLELNER
FLT4-L RRSLEPATLPQ COAANKTCPT NYMNNHICR CLAQEDFMFS SDAGDDSTDG 300
251
PDGF-A
PDGF-B A.....
PLGF
VEGF
FLT4-L FHDICGNKE LDEETCQCVC RAGLRPASC G PKELDRNSC QCVCKNKLFP 350
301
PDGF-A
PDGF-B
PLGF
VEGF
FLT4-L SQCGANREFD ENTQCQCCKR TCPRNQPLNP GKCACTES PQKLLKGKK 395
351
PDGF-A
PDGF-B
PLGF
VEGF
FLT4-L FHHQTCSCYR RPCTNRQKAC EPGFSYSSEV CRCVPSYWK R PQMS

FIGURE 10B

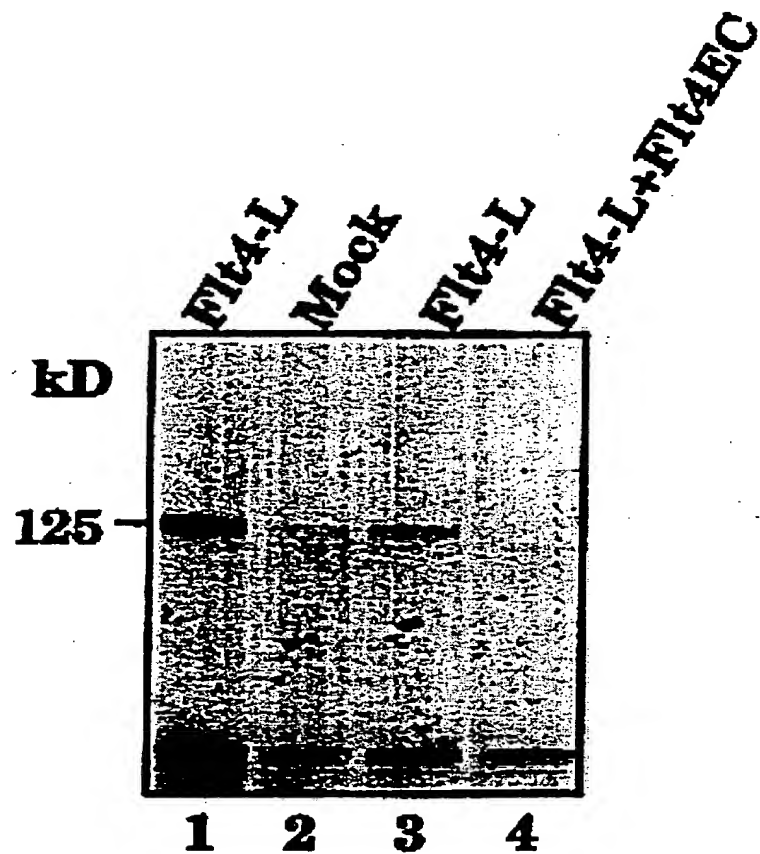


FIGURE II

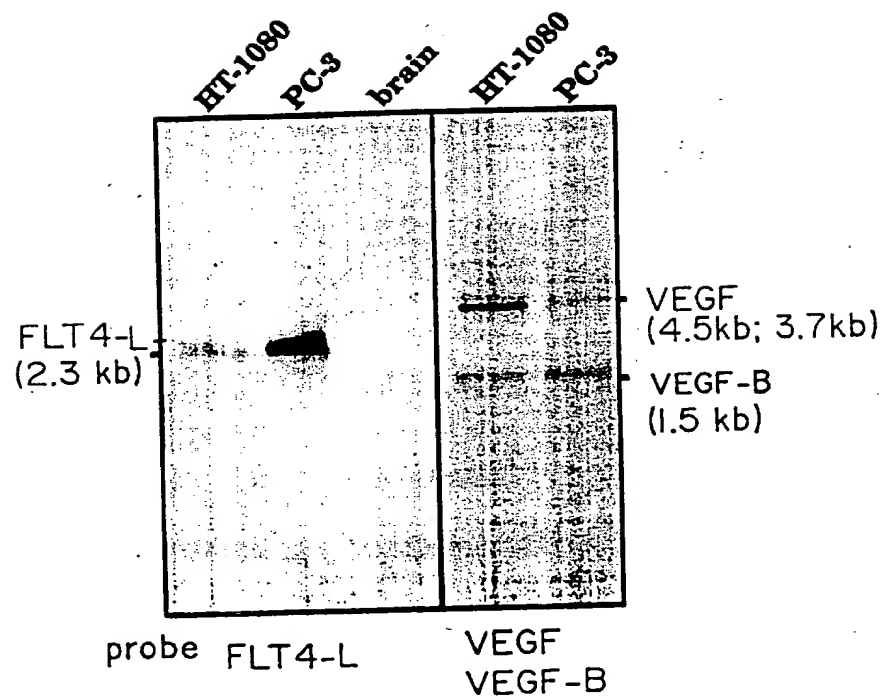


FIGURE 12

FTT4 LIGAND AND METHODS OF USE

FIELD OF THE INVENTION

The present invention generally relates to the field of genetic engineering and more particularly to growth factors for endothelial cells and growth factor genes.

BACKGROUND OF THE INVENTION

Developmental growth, the remodelling and regeneration of adult tissues as well as solid tumor growth, can only occur when accompanied by blood vessel formation. Angioblasts and hematopoietic precursor cells differentiate from the mesoderm and form the blood islands of the yolk sac and the primary vascular system of the embryo. The development of blood vessels from these early (in situ) differentiating endothelial cells is termed vasculogenesis. Major embryonic blood vessels are believed to arise via vasculogenesis, whereas the formation of the rest of the vascular tree is thought to occur as a result of vascular sprouting from pre-existing vessels, a process called angiogenesis, Risau, et al., *Devel. Biol.*, 125: 441-450 (1988).

Endothelial cells give rise to several types of functionally and morphologically distinct vessels. When organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases. Upon angiogenic stimulation, endothelial cells may re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their tissue environment. Endothelial cells undergoing angiogenesis degrade the underlying basement membrane and migrate, forming capillary sprouts that project into the perivascular stroma. Ausprunk, et al., *Micronasc. Rev.*, 14: 51-65 (1977). Angiogenesis during tissue development and regeneration depends on the tightly controlled processes of endothelial cell proliferation, migration, differentiation and survival. Dysfunction of the endothelial cell regulatory system is a key feature of many diseases. Most importantly, tumor growth and metastasis have been shown to be angiogenesis dependent. Folkman, et al., *J. Biol. Chem.*, 267:10931-10934 (1992).

Key signals regulating cell growth and differentiation are mediated by polypeptide growth factors and their transmembrane receptors, many of which are tyrosine kinases. Autophosphorylated peptides within the tyrosine kinase insert and carboxyl-terminal sequences of activated receptors are commonly recognized by kinase substrates involved in signal transduction for the readjustment of gene expression in responding cells. Several families of receptor tyrosine kinases have been characterized. Van der Geer, et al., *Ann. Rev. Cell Biol.*, 10:251-337 (1994). The major growth factors and receptors transducing angiogenic stimuli are schematically shown in FIG. 1.

Fibroblast growth factors are also known to be involved in the regulation of angiogenesis. They have been shown to be mitogenic and chemotactic for cultured endothelial cells. Fibroblast growth factors also stimulate the production of proteases, such as collagenases and plasminogen activators, and induce tube formation by endothelial cells. Saksela, et al., *Ann. Rev. Cell Biol.*, 4:93-126 (1988). There are two general classes of fibroblast growth factors, FGF-1 and FGF-2, both of which lack conventional signal peptides. Both types have an affinity for heparin and FGF-2 is bound to heparin sulfate proteoglycans in the subendothelial extracellular matrix from which it may be released after injury. Heparin potentiates the stimulation of endothelial cell proliferation by angiogenic FGFs, both by protecting against

denaturation and degradation and dispersing the FGFs. Cultured endothelial cells express the FGF-1 receptor but no significant levels of other high-affinity fibroblast growth factor receptors.

Among other ligands for receptor tyrosine kinases, the platelet derived growth factor, PDGF-BB, has been shown to be weakly angiogenic in the chick chorioallantoic membrane. Risau, et al., *Growth Factors*, 7:261-266 (1992). Transforming growth factor α (TGF α) is an angiogenic factor secreted by several tumor cell types and by macrophages. Hepatocyte growth factor (HGF), the ligand of the c-met proto-oncogene-encoded receptor, is also strongly angiogenic.

Recent evidence shows that there are endothelial cell specific growth factors and receptors that may be primarily responsible for the stimulation of endothelial cell growth, differentiation and certain differentiated functions. The best studied of these is vascular endothelial growth factor (VEGF), a member of the PDGF family. Vascular endothelial growth factor is a dimeric glycoprotein of disulfide-linked 23 kDa subunits, discovered because of its mitogenic activity toward endothelial cells and its ability to induce vessel permeability (hence its alternative name vascular permeability factor). Other reported effects of VEGF include the mobilization of intracellular calcium, the induction of plasminogen activator and plasminogen activator inhibitor-1 synthesis, stimulation of hexose transport in endothelial cells, and promotion of monocyte migration in vitro. Four VEGF isoforms encoded by distinct mRNA splice variants appear to be equally capable of stimulating mitogenesis in endothelial cells. However, each has a different affinity for cell surface proteoglycans, which behave as low affinity receptors for VEGF. The 121 and 165 amino acid isoforms of VEGF are secreted in a soluble form, whereas the isoforms of 189 and 206 amino acid residues remain cell surface associated and have a strong affinity for heparin.

The pattern of VEGF expression suggests its involvement in the development and maintenance of the normal vascular system and in tumor angiogenesis. During murine development, the entire 7.5 day post-coital (p.c.) endoderm expresses VEGF and the ventricular neuroectoderm produces VEGF at the capillary ingrowth stage. Breier, et al., *Development*, 114:521-523 (1992). On day two of quail development, the vascularized area of the yolk sac as well as the whole embryo show expression of VEGF. In addition, epithelial cells next to fenestrated endothelia in adult mice show persistent VEGF expression, suggesting a role in the maintenance of this specific endothelial phenotype and function.

Two high affinity receptors for VEGF have been characterized. These are VEGFR-1/Flt-1 (fms-like tyrosine kinase-1) and VEGFR-2/Kdr/Flk-1 (kinase insert domain containing receptor/fetal liver kinase-1). Those receptors are classified in the PDGF-receptor family, but they have seven rather than five immunoglobulin-like loops in their extracellular domain and they possess a longer kinase insert than normally observed in this family. The expression of VEGF receptors occurs mainly in vascular endothelial cells, although some may be present on monocytes and melanoma cells. Only endothelial cells have been reported to proliferate in response to VEGF and endothelial cells from different sources show different responses. Thus, the signals mediated through VEGFR-1 and VEGFR-2 appear to be cell type specific.

The Flt4 receptor tyrosine kinase is closely related in structure to the products of the VEGFR-1 and VEGFR-2

genes. Despite this similarity, the mature form of Flt4 differs from the VEGF receptors in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola et al., *Cancer Res.*, 52:5738-5743 (1992). The 4.5 and 5.8 kb Flt-4 mRNAs encode polypeptides which differ in their C-termini due to the use of alternative 3' exons. The VEGFs do not show specific binding to Flt4 or induce its autophosphorylation.

Expression of Flt4 appears to be more restricted than expression of VEGFR-1 or VEGFR-2. The expression of Flt4 first becomes detectable by in situ hybridization in the angioblasts of head mesenchyme, the cardinal vein, and extraembryonically in the allantois of 8.5 day p.c. mouse embryos. In 12.5 day p.c. embryos the Flt-4 signal is observed in developing venous and presumptive lymphatic endothelia, but arterial endothelia appear negative. During later stages of development, Flt4 mRNA becomes restricted to developing lymphatic vessels. Only the lymphatic endothelia and some high endothelial venules express Flt4 mRNA in adult human tissues and increased expression occurs in lymphatic sinuses in metastatic lymph nodes and in lymphangioma. These results support the theory of the venous origin of lymphatic vessels.

SUMMARY OF THE INVENTION

The present invention provides a ligand for the Flt4 receptor tyrosine kinase. In a preferred embodiment, the ligand comprises a fragment of the amino acid sequence shown in SEQ ID NO: 33 which specifically binds to the Flt4 receptor tyrosine kinase.

The present invention also provides a precursor of an Flt4 ligand, wherein the precursor comprises the amino acid sequence shown in SEQ ID NO: 33. The precursor is proteolytically cleaved upon expression to produce an approximately 23 kD peptide which is the Flt4 ligand. In a preferred embodiment of the invention, an Flt4 ligand is provided which is the cleavage product of the precursor peptide shown in SEQ ID NO: 33 and which has a molecular weight of approximately 23 kD under reducing conditions. The Flt4 ligand comprises approximately the first 180 amino acids shown in SEQ ID NO: 33.

Also in a preferred embodiment, nucleic acids encoding an Flt4 ligand precursor are presented. Due to the degeneracy of the genetic code, numerous such coding sequences are possible, each having in common the coding of the amino acid sequence shown in SEQ ID NO: 33, or portions thereof. Ligand precursors according to the invention, when expressed in an appropriate host cell, produce, via cleavage, a peptide which binds specifically to the Flt4 receptor tyrosine kinase. The nucleotide sequence encoding the Flt4 ligand is within the nucleotide sequence shown in SEQ ID NO: 32.

The present invention also provides a cell line which produces an Flt4 ligand. The ligand may be purified and isolated directly from the cell culture medium. Also provided are vectors comprising DNA encoding the Flt4 ligand and host cells comprising the vectors. Vectors are capable of expressing the Flt4 ligand under the control of appropriate promoters and other control sequences.

Ligands according to the invention may be labeled with a detectable label and used to identify their corresponding receptors in situ. Antibodies, both monoclonal and polyclonal, may be made against a ligand of the invention according to standard techniques in the art. Such antibodies may be used in diagnostic applications to monitor angiogenesis, vascularization, lymphatic vessels and their

disease states, wound healing, or certain hematopoietic or leukemia cells or they may be used to block or activate the Flt4 receptor. Labeled Flt4 ligand and anti-Flt4 ligand antibodies may be used as imaging agents in the detection of lymphatic vessels, high endothelial venules, and Flt4 receptors expressed in histochemical tissue sections. The ligand or antibody may be covalently or non-covalently coupled to a suitable supermagnetic, paramagnetic, electron dense, echogenic, or radioactive agent for imaging. Other, non-radioactive labels, such as biotin and avidin may also be used.

The present invention also provides diagnostic and clinical applications for claimed ligands. In a preferred embodiment, Flt4 ligands or precursors of the invention are used to accelerate angiogenesis e.g. during wound healing or to promote the endothelial functions of lymphatic vessels. Ligands may be applied in any suitable manner using an appropriate pharmaceutically-acceptable vehicle. Ligands may also be used to quantify future metastatic risk by assaying biopsy material for the presence of active receptors or ligands in a binding assay or kit using detectably-labeled ligand. An Flt4 ligand according to the invention may also be used to promote re-growth or permeability of lymphatic vessels in, for example, organ transplant patients. Ligands according to the invention may also be used to treat or prevent inflammation, edema, aplasia of the lymphatic vessels, lymphatic obstruction, elephantiasis, and Milroy's disease. Finally, Flt4 ligands may be used to stimulate lymphocyte production and maturation and to promote or inhibit trafficking of leukocytes between tissues and lymphatic vessels or to affect migration in and out of the thymus.

Inhibitors of the Flt4 ligand may be used to control endothelial cell proliferation and lymphangiomas. For example, such inhibitors may be used to arrest metastatic growth or spread or to control other aspects of endothelial cell expression and growth. Inhibitors include antibodies, antisense oligonucleotides, and peptides which block the Flt4 receptor.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic diagram showing major endothelial cell receptor tyrosine kinases and growth factors involved in vasculogenesis and angiogenesis.

FIGS. 2A and 2B show schematically the construction of the pLTRFlt4I expression vector.

FIG. 3 shows schematically the construction of the baculovirus vector encoding a secreted soluble Flt4EC domain.

FIG. 4 shows results of stimulation of Flt4 autophosphorylation by conditioned medium from PC-3 cell cultures.

FIGS. 5A-5C show that the tyrosyl phosphorylated polypeptide of Flt4-transfected cells stimulated with PC-3 conditioned medium is the 125 kD Flt4 polypeptide.

FIGS. 6A and 6B show Western analysis of the Flt4 ligand activity isolated from PC-3 conditioned medium.

FIG. 7 shows results of gel electrophoresis of fractions from the Western analysis of Flt4 ligand isolated from PC-3 conditioned medium.

FIG. 8 shows results of Western analysis of Flt4 autophosphorylation induced by either the Flt4 ligand, VEGF, or PlGF.

FIGS. 9A through 9C show the nucleotide and deduced amino acid sequence of the coding portion of Flt4 ligand cDNA.

FIGS. 10A and 10B show a comparison of the deduced amino acid sequences of PDGF-A, -B, two PlGF isoforms, four VEGF isoforms and Flt4 ligand.

FIG. 11 shows the stimulation of autophosphorylation of the Flt4 receptor by conditioned medium from cells transfected with the Flt4-L expression vector

FIG. 12 shows Northern blotting analysis of Flt4-L mRNA in tumor cell lines.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to novel growth factors which are ligands for the Flt4 receptor tyrosine kinase. Claimed ligands are members of a family of platelet-derived growth factors/vascular endothelial growth factors which promote mitosis and proliferation of vascular endothelial cells and/or mesodermal cells. Ligands recognizing the Flt4 receptor tyrosine kinase were purified from a PC-3 prostatic adenocarcinoma cell line (ATCC CRL1435). When applied to a population of cells expressing the Flt4 receptor, ligands of the invention stimulate autophosphorylation, resulting in receptor activation. The invention also provides inhibitors of the Flt4 receptor, including antibodies directed against the receptor. A ligand according to the invention may be coexpressed as a larger precursor which is cleaved to produce the ligand. A coexpressed region in some cases results from alternative splicing of RNA of the ligand gene. Such a co-expressed region may be a function of the particular expression system used to obtain the ligand. The skilled artisan understands that in recombinant production of proteins, additional sequence may be expressed along with a functional peptide depending upon the particular recombinant construct used to express the protein, and subsequently removed to obtain the desired ligand. In some cases the recombinant ligand can be made lacking certain residues of the endogenous/natural ligand. Moreover, it is well-known in that conservative replacements may be made in a protein which do not alter the function of the protein. Accordingly, it is anticipated that such alterations are within the scope of the claims. It is intended that the precursor sequence shown in SEQ ID NO: 33 is capable of stimulating the Flt4 ligand without any further processing in a manner similar to that in which VEGF stimulates its receptor in its unprocessed form.

The following Examples illustrate preferred embodiments of the invention, wherein the isolation, characterization, and function of Flt4 ligands according to the invention is shown.

EXAMPLE 1

Production of pLTR[Flt4] Expression Vector

Construction of the LTR-Flt4 vector is schematically shown in FIGS. 2A and 2B. The full-length Flt4s cDNA (Genbank Accession No. X68203) was assembled by first subcloning the S2.5 fragment, reported in Pajusola et al., *Cancer Res.* 52:5738-5743 (1992), incorporated by reference herein, containing base pairs 56-2534 of the Flt4s into the EcoRI site of the pSP73 vector (Promega, Madison, Wis.).

Since cDNA libraries used for screening of Flt4 cDNAs did not contain its most 5' protein-coding sequences, inverse PCR was used for the amplification of the 5' end of Flt4 corresponding to the first 12 amino acid residues (MQRGAALCLRI.W). PolyA+RNA was isolated from the HEL cells and double-stranded cDNA copy was synthesized using the Amersham cDNA Synthesis System Plus kit and a gene specific primer: 5'-TGTCCTCGCTGCTTGTCT-3' (SEQ ID NO: 1), which was located 195 bp downstream of the 5' end of clone S2.5. Double stranded cDNA was treated

with T4 DNA polymerase to blunt the ends and cDNA was purified with Centricon 100 (Amicon Inc., Beverly, Mass.). Circularization was made in a total volume of 150 μ l. The reaction mixture contained ligation buffer, 5% PEG-8000, 1 mM DTT and 8 U of T4 DNA ligase (New England Biolabs). Ligation was carried out at 16° C. for 16 hours. Fifteen μ l of this reaction mix was used in a standard 100 μ l PCR reaction containing 100 ng of specific primers including SacI and PstI restriction sites, present in this segment of the Flt4 cDNA, and 1 unit of Taq DNA polymerase (Perkin Elmer Cetus). Two rounds of PCR were performed using 33 cycles (denaturation at 95° C. for 1 minute, annealing at 55° C. for 2 minutes and elongation at 72° C. for 4 minutes). The PCR mixture was treated sequentially with the SacI and PstI restriction enzymes and after purification with MagicPCR Preps (Promega) DNA fragments were subcloned into the pGEM3Zf(+) vector for sequencing. The sequence obtained corresponds to the 5' end of the Flt4s cDNA clone deposited in the Genbank Database as Accession No. X68203.

The sequence encoding the first 12 amino acid residues was added to the expression construct by ligating an SphI digested PCR fragment amplified using reverse transcription-PCR of polyA+RNA isolated from the HEL cells using the oligonucleotides 5'-ACATGCATGC CAC-CATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG GACTCCTGGA-3' (SEQ ID NO: 2) (forward primer, SphI site underlined, the translational start codon marked in bold follows an optimized Kozak consensus sequence Kozak, *Nucl. Acids Res.* 15: 8125-8148, 1987) and 5'-ACATGCATGC CCGCCCGGT CATCC-3' (SEQ ID NO: 3) (reverse primer, SphI site underlined) to the 5' end of the S2.5 fragment, thus replacing unique SphI fragment of the S2.5 plasmid. The resulting vector was digested with EcoRI and ClaI and ligated to a 138 bp PCR fragment amplified from the 0.6 kb EcoRI fragment (base pairs 3789 to 4416 in the Genbank X68203 sequence) which encodes the 3' end of Flt4s shown in FIG. 1 of Pajusola et al., *Cancer Res.* 52:5738-5743, 1992, using the oligonucleotides 5'-CGGAATTCCTCC CATGACCCCA AC-3' (SEQ ID NO: 4) (forward, EcoRI site underlined) and 5'-CC ATCGATGG ATCTACTCT AAGCCGCTTT CTT-3' (SEQ ID NO: 5) (reverse, ClaI site underlined). The coding domain was completed by ligation of the 1.2 kb EcoRI fragment (base pairs 2535-3789 of sequence X68203) into the above construct. The complete cDNA was subcloned as a HindIII-ClaI (blunted) fragment (this ClaI site was also included in the 3' primer used to construct the 3' end of the coding sequence) to the pLTRpoly expression vector reported in Mäkelä et al., *Gene*, 118: 293-294 (1992) (Genbank accession number X60280), incorporated by reference herein, using its HindIII-Acc I (blunted) restriction sites.

The long form of Flt4 was produced by replacing the 3'-end of the short form as follows: The 3' region of the Flt4 cDNA was PCR-amplified using a gene specific and a pGEM 3Z vector specific (SP6 promoter) oligonucleotide 5'-ATTAGGTGACACTATA-3' (SEQ ID NO: 6) as reverse and forward primers, respectively, and an Flt4 cDNA clone containing a 495 bp EcoRI fragment extending downstream of the EcoRI site at nucleotide 3789 of the Genbank X68203 sequence (the sequence downstream of this EcoRI site is deposited as the Flt4 long form 3' sequence having Genbank accession number S66407). The gene specific oligonucleotide contained a BamHI restriction site located right after the end of the coding region. The sequence of that (reverse primer) oligonucleotide was 5'-CCATCGAT GGATCCCGATGCTGCTTAGTAGCTGT-3' (SEQ ID NO:

7) (BamHI site is underlined). The PCR product was digested with EcoRI and BamHI and transferred in frame to LTRFlt4s vector fragment from which the coding sequences downstream of the EcoRI site at base pair 2535 (see sequence X68203) had been removed by EcoRI-BamHI digestion. Again, the coding domain was completed by ligation of the 1.2 kb EcoRI fragment (base pairs 2535-3789 of sequence X68203) back into the resulting construct.

EXAMPLE 2

Production and Analysis of Flt4 Transfected Cells

NIH3T3 cells (60% confluent) were cotransfected with 5 µg of the pLTRFlt4 construct and 0.25 µg of the pSV2neo vector (ATCC) containing the neomycin phosphotransferase gene, using the DOTAP liposome-based transfection reagents (Boehringer Mannheim, Mannheim, Germany). One day after the transfection the cells were transferred into selection media containing 0.5 mg/ml geneticin (GIBCO, Grand Island, N.Y.). Colonies of geneticin-resistant cells were isolated and analysed for expression of the Flt4 proteins. Cells were lysed in boiling lysis buffer containing 3.3% SDS (sodium dodecyl sulphate), 125 mM Tris, pH 6.8. Protein concentrations of the samples were measured by the BCA method (Pierce, Rockford, Ill.). About 50 µg protein of each lysate was analysed for the presence of Flt4 by 6% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using antisera against the carboxyl terminus of Flt4 and the ECL method (Amersham).

For production of anti-Flt4 antiserum the Flt4 cDNA fragment encoding the 40 carboxyterminal amino acid residues of the short form: NH₂-PMTPTTYKG SVDN-QTDSGM VLASEEFEQI ESRHRQESGFR-COOH (SEQ ID NO: 8) was cloned as a 657 bp EcoRI-fragment into the pGEX-11T bacterial expression vector (Pharmacia) in frame with the glutathione-S-transferase coding region. The resulting GST-Flt4S fusion protein was produced in *E. coli* and purified by affinity chromatography using a glutathione-Sepharose 4B column. The purified protein was lyophilized, dissolved in phosphate buffered saline (PBS), mixed with Freund's adjuvant and used for immunization of rabbits at biweekly intervals using methods standard in the art (Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988). Antisera were used after the fourth booster immunization for immunoprecipitation of Flt4 from the transfected cells and clones expressing Flt4 were used for ligand stimulation analysis.

EXAMPLE 3

Construction of a Flt4 EC Baculovirus Vector and Expression and Purification of its Product

The construction of an Flt4 extracellular domain (EC) baculovirus vector is schematically shown in FIG. 3. The Flt4-encoding cDNA has been prepared in both a long form and a short form, each being incorporated in a vector under control of the Mooney murine leukemia virus LTR promoter. The nucleotide sequence of the short form of the Flt4 receptor is available on the Genbank database as Accession No. X68203 and the specific 3' segment of the long form cDNA is available as Accession No. S66407.

The ends of a cDNA segment encoding Flt4 extracellular domain (EC) were modified as follows: The 3' end of Flt4 cDNA sequence (Genbank Accession Number X68203) which encodes the extracellular domain was amplified using primer 1116 5'-CTGGAGTTCGACTTGCGCGACT-3' (SEQ

ID NO: 9, SalI site underlined) and primer 1315 5'-CGC GGATCCCTAGTGATGGTGATGGTGATGCTACCTTC GATCATG CTGCCCTTAT CCTC-3' (SEQ ID NO: 10, BamHI site underlined). The sequence complementary to that of primer 1315 continues after the Flt4 reading frame and encodes 6 histidine residues for binding to a Ni-NTA column (Qiagen, Hilden, Germany) followed by a stop codon, and an added Bam HI site. The amplified fragment was digested with SalI and BamHI and used to replace a unique SalI-BamHI fragment in the LTRFlt4 vector shown in FIG. 3. The SalI-BamHI fragment that was replaced encodes the Flt4 transmembrane and cytoplasmic domains.

The 5' end without the Flt4 signal sequence encoding region was amplified by PCR using the primer 1335 5'-CCC AAGCTTGGATCCCAAGTGGCTACTCCATGACC-3' (SEQ ID NO: 11) (the primer contains added HindIII (AAGCTT) and BamHI (GGATCC) restriction sites, which are underlined) and primer 1332 5'-GTTGCCCTGTGATGTGCACCA-3' (SEQ ID NO: 12). The amplified fragment was digested with HindIII and SphI (the HindIII site (AAGCTT) is underlined in primer 1335 and the SphI site is within the amplified region of the Flt4 cDNA). The resulting HindIII-SphI fragment was used to replace a HindIII-SphI fragment in the modified LTRFlt4 vector described immediately above (the HindIII site is in the 5' junction of the Flt4 insert with the pLTRpoly portion of the vector, the SphI site is in Flt4 cDNA). The resulting Flt4EC insert was then ligated as a BamHI fragment into the BamHI site in the pVTBac plasmid as disclosed in Tessier et al., *Gene* 98: 177-183 (1991), incorporated by reference herein. The orientation was confirmed to be correct by partial sequencing so that the open reading frame of the signal sequence-encoding portion of the vector continued in frame with the Flt4 sequence. That construct was transfected together with the baculovirus genomic DNA into SF-9 cells by lipofection. Recombinant virus was purified, amplified and used for infection of High-Five cells (Invitrogen, San Diego, Calif.) using methods standard in the art. The Flt4 extracellular domain was purified from the culture medium of the infected High-Five cells using Ni-NTA affinity chromatography according to manufacturer's instructions (Qiagen) for binding and elution of the 6xHis tag encoded in the COOH-terminus of the recombinant Flt4 extracellular domain.

EXAMPLE 4

Isolation of Flt4 Ligand from Conditioned Media

An Flt4 ligand according to the invention was isolated from conditioned media from PC-3 prostatic adenocarcinoma cell line CRL1435 from the American Type Culture Collection and cultured as instructed by the supplier in Ham's F-12 Nutrient mixture (GIBCO) containing 7% fetal calf serum. In order to prepare the conditioned media, confluent PC-3 cells were cultured for 7 days in Ham's F-12 Nutrient mixture (GIBCO) in the absence of fetal bovine serum. Medium was then cleared by centrifugation at 10,000 g for 20 minutes. The medium was then screened to determine its ability to induce tyrosine phosphorylation of Flt4 by exposure to NIH3T3 cells which had been transfected with Flt4-encoding cDNA using the pLTRFlt4 vector. For receptor stimulation experiments, subconfluent NIH3T3 cells were starved overnight in serum-free DMEM medium (GIBCO) containing 0.2% BSA. The cells were stimulated with the conditioned media for 5 minutes, washed twice with cold PBS containing 100 µM vanadate and lysed in RIPA buffer (10 mM Tris pH 7.5, 50 mM NaCl, 0.5% sodium

deoxycholate, 0.5% Nonidet P40 (BDH, Poole, England), 0.1% SDS, 0.1 U/ml Aprotinin (Boehringer Mannheim), 100 uM vanadate) for receptor immunoprecipitation analysis. The lysates were centrifuged for 20 minutes at 15,000xg. The supernatants were incubated for 2 hours on ice with 3 ul of the antiserum against the Flt4 C-terminus described in Example 2 and also in Pajusola, et al. *Oncogene* 8: 2931-2937, (1993), incorporated by reference herein.

After a 2 hour incubation in the presence of anti-Flt4 antiserum, protein A-Sepharose (Pharmacia) was added and incubation was continued for 45 minutes with rotation. The immunoprecipitates were washed three times with the immunoprecipitation buffer and twice with 10 mM Tris, pH 7.5 before analysis in SDS-PAGE. Polypeptides were transferred to nitrocellulose and analyzed by Western blotting using Flt4- or phosphotyrosine-specific antisera and the ECL method (Amersham International, Buckinghamshire, England). Anti-phosphotyrosine monoclonal antibodies (anti-PTyr, PY20) were purchased from Transduction Laboratories (Lexington, Ky.). In some cases, the filters were restained with a second antibody after stripping. The stripping of the filters was done for 30 minutes at 50° C. in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7 with occasional agitation.

As shown in FIG. 4, the PC-3 cell conditioned medium stimulated tyrosine phosphorylation of a 125 kD polypeptide when Flt4 expressing NIH3T3 cells were treated with the indicated preparations of media, lysed, and the lysates were immunoprecipitated with anti-Flt4 antiserum followed by SDS-PAGE, Western blotting, and staining using anti-PTyr antibodies. The resulting band was weakly phosphorylated upon stimulation with unconcentrated PC-3 conditioned medium (lane 2). The 125 kD band comigrated with the tyrosine phosphorylated, processed form of the mature Flt4 from pervanadate-treated cells (compare lanes 2 and 7 of FIG. 4, see also FIG. 5A). Comigration was confirmed upon restaining with anti-Flt4 antibodies as is also shown in FIG. 5A (panel on the right). In order to show that the 125 kD polypeptide is not a non-specific component of the conditioned medium reactive with anti-phosphotyrosine antibodies, 15 ul of conditioned medium was separated by SDS-PAGE, blotted on nitrocellulose and the blot was stained with anti-PTyr antibodies. No signal was obtained (FIG. 5B). Also, unconditioned medium failed to stimulate Flt4 phosphorylation, as shown in FIG. 4, lane 1.

As shown in FIG. 4, lane 3, stimulating activity was considerably increased when the PC-3 conditioned medium was concentrated four-fold using a Centricon-10 concentrator (Amicon). FIG. 4, lane 4, shows that pretreatment of the concentrated PC-3 conditioned medium with 50 ul of the Flt4 extracellular domain coupled to CNBr-activated sepharose CL-4B (Pharmacia; about 1 mg of Flt4 EC domain/ml sepharose resin) completely abolished Flt4 tyrosine phosphorylation. Similar pretreatment of the conditioned medium with unsubstituted sepharose CL-4B did not affect stimulatory activity, as shown in FIG. 4, lane 5. Also, the flow through obtained after concentration, which contained proteins of less than 10,000 molecular weight, did not stimulate Flt4 phosphorylation, as shown in FIG. 4, lane 6.

The foregoing data show that PC-3 cells produce a ligand which binds to the extracellular domain of Flt4 and activates this receptor.

EXAMPLE 5

Purification of the Flt4 Ligand

The ligand expressed by PC-3 cells as characterized in Example 3 was purified and isolated using a recombinant by produced Flt4 extracellular domain in affinity chromatography.

Two harvests of serum-free conditioned medium, comprising a total of 8 L were collected from 500 confluent 15 cm diameter culture dishes containing confluent layers of PC-3 cells. The conditioned medium was clarified by centrifugation at 10,000xg and concentrated 80-fold using an Ultrasette Tangential Flow Device (Filtron, Northborough, Mass.) with a 10 kD cutoff Omega Ultrafiltration membrane according to the manufacturer's instruction. Recombinant Flt4 extracellular domain was expressed in a recombinant baculovirus cell system and purified by affinity chromatography on Ni-agarose (Ni-NTA affinity column obtained from Qiagen). The purified extracellular domain was coupled to CNBr-activated Sepharose CL-4B at a concentration of 5 mg/ml and used as an affinity matrix for ligand affinity chromatography.

Concentrated conditioned medium was incubated with 2 ml of the recombinant Flt4 extracellular domain-Sepharose affinity matrix in a rolling tube at room temperature for 3 hours. All subsequent purification steps were at +4° C. The affinity matrix was then transferred to a column (Pharmacia) with an inner diameter of 15 mm and washed successively with 100 ml of PBS and 50 ml of 10 mM Na-phosphate buffer (pH 6.8). Bound material was eluted step-wise with 100 mM glycine-HCl, successive 6 ml elutions having pHs of 4.0, 2.4, and 1.9. Several 2 ml fractions of the eluate were collected in tubes containing 0.5 ml 1 M Na-phosphate (pH 8.0). Fractions were mixed immediately and dialysed in 1 mM Tris-HCl (pH 7.5). Aliquots of 75 ul each were analyzed for their ability to stimulate tyrosine phosphorylation of Flt4. The ultrafiltrate, 100 ul aliquots of the concentrated conditioned medium before and after ligand affinity chromatography, as well as 15-fold concentrated fractions of material released from the Flt4 extracellular domain-Sepharose matrix during the washings were also analyzed for their ability to stimulate Flt4 tyrosine phosphorylation.

As shown in FIGS. 6A and 6B, lane 3, the concentrated conditioned medium induced prominent tyrosine phosphorylation of Flt4 in transfected NIH3T3 cells overexpressing Flt4. This activity was not observed in conditioned medium taken after medium was exposed to the Flt4 Sepharose affinity matrix described above (lane 4). The specifically-bound Flt4-stimulating material was retained on the affinity matrix upon washes in PBS, 10 mM Na-phosphate buffer (pH 6.8), and at pH 4.0 (lanes 5-7, respectively), and it was eluted in the first two 2 ml aliquots at pH 2.4 (lanes 8 and 9). A further decrease of the pH of the elution buffer did not cause release of additional Flt4-stimulating material (lane 11).

Small aliquots of the chromatographic fractions were concentrated in a SpeedVac concentrator (Savant, Farmingdale, N.Y.) and subjected to SDS-PAGE under reducing conditions with subsequent silver staining of the gel. As shown in FIG. 7, the major polypeptide, having a molecular weight of approximately 23 kD (reducing conditions), was detected in the fractions containing Flt4 stimulating activity (corresponding to lanes 8 and 9 in FIG. 6). That polypeptide was not found in the other chromatographic fractions. On the other hand, all other components detected in the two active fractions were also distributed in the starting material and in small amounts in the other washing and elution steps after their concentration. Similar results were obtained in three independent affinity purifications, indicating that the 23 kD polypeptide specifically binds to Flt4 and induces its tyrosine phosphorylation. Fractions containing the 23 kD polypeptide were combined, dried in a SpeedVac concentrator and subjected to SDS-PAGE in a 12.5% gel. The proteins from the gel

were then electroblotted to Immobilon-P (PVDF) transfer membrane (Millipore, Marlborough, Mass.) and visualized by staining of the blot with Coomassie blue R-250. The region containing only the stained 23 kD band was cut from the blot and was subjected to N-terminal amino acid sequence analysis in a Prosite Protein Sequencing System (Applied Biosystems, Foster City, Calif.). The data were analyzed using a 610A Data Analysis System (Applied Biosystems). Analysis revealed a single N-terminal sequence of NH₂-XETIKFAAAHYNTEILK-COOH (SEQ ID NO: 13).

EXAMPLE 6

Construction of PC-3 Cell cDNA Library in a Eukaryotic Expression Vector

Poly-A+RNA was isolated from five 15 cm diameter confluent dishes of PC-3 cells by a single step method using oligo(dT) (Type III, Collaborative Research) cellulose affinity chromatography (Sambrook et al., Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989). The yield was 70 µg. Six µg of the poly-A+RNA was used to prepare an oligo(dT)-primed cDNA library in the mammalian expression vector pcDNA1 and the Librarian kit of Invitrogen according to the instructions included in the kit. The library was estimated to contain about 10⁶ independent recombinants with an average insert size of approximately 1.8 kb.

EXAMPLE 7

Amplification of the Unique Nucleotide Sequence Encoding the Flt4 Ligand

Degenerate oligonucleotides were designed based on the N-terminal amino acid sequence of the isolated Flt4 ligand and were used as primers in a polymerase chain reaction (PCR) to amplify cDNA encoding the Flt4 ligand from a PC-3 cell library.

The PCR was carried out using 1 µg of DNA from the amplified PC-3 cDNA library and a mixture of sense-strand primers comprising 5'-GCAGARGARACNATHAA-3' (SEQ ID NO: 14) (wherein R is A or G, N is A, G, C or T and H is A, C or T), encoding amino acid residues 2-6 (EETIK, SEQ ID NO: 15) and antisense-strand primers 5'-GCAYTTNARIATYTCNGT-3' (SEQ ID NO: 16) (wherein Y is C or T and D is A, G or T), corresponding to amino acid residues 14-18 (TEILK, SEQ ID NO: 17). Three extra nucleotides (GCA) were added to the 5'-terminus of each primer to increase annealing stability. Two successive PCR runs were carried out using 1 U per reaction of DynaZyme, a thermostable DNA polymerase (F-500L, Finnzymes), in a buffer supplied by the manufacturer (10 mM Tris-HCl, pH 8.8 at 25° C., 1.5 mM MgCl₂, 50 mM KCl, 0.1% Triton-X100) at an extension temperature of 72° C. The first PCR run was carried out for 43 cycles. The first three cycles were run at annealing temperature 33° C. for 2 minutes and the remaining cycles were run at 42° C. for 1 minute.

The region of the gel containing a weak band of the expected size (57 bp) was cut out from the gel and eluted. The eluted material was reamplified for 30 cycles using the same primer pairs described above at 42° C. for 1 minute. The amplified fragment was cloned into a pCR II vector (Invitrogen) using the TA cloning kit (Invitrogen) and sequenced using the radioactive dideoxynucleotide sequencing method of Sanger. Six clones were analysed and all

contained the sequence encoding the expected peptide (amino acids 2-18 of the Flt4 ligand precursor). Nucleotide sequence spanning the region from the third nucleotide of codon 6 to the third nucleotide of codon 13 (the extension region) was identical in all six clones: 5'-ATTGCTGCGAGCAGCACTACAAC-3' (SEQ ID NO: 18) and thus was considered to represent an amplified product from the unique sequence encoding part of the amino terminus of the Flt4 ligand.

EXAMPLE 8

Amplification of the 5'-end of the cDNA Encoding the Flt4 Ligand

Based on the unique nucleotide sequence encoding the N-terminus of the isolated Flt4 ligand, two pairs of nested primers were designed to amplify in two subsequent PCR-reactions the complete 5'-end of the corresponding cDNAs from 1 µg of DNA from the above-described PC-3 cDNA library. First, amplification was performed with primer 5'-TCNGTGTGTAGTGTGCTG-3' (SEQ ID NO: 19) which is the antisense-strand primer corresponding to amino acid residues 9-15 (AAHYNTE, SEQ ID NO: 20) and sense-strand primer 5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO: 21) corresponding to the T7 RNA promoter of the pcDNA1 vector used for construction of the library. "Touchdown" PCR was used as disclosed in Don, et al., *Nucl. Acids Res.*, 19: 4008 (1991), incorporated by reference herein. The annealing temperature of the two first cycles was 62° C. and subsequently the annealing temperature was decreased in every other cycle by 1° C. until a final temperature of 53° C. was reached, at which temperature 16 additional cycles were carried out. Annealing time as 1 minute and extension at each cycle was conducted at 72° C. for 1 minute. Multiple amplified DNA fragments were obtained in the first reaction. The products of the first amplification (1 µl of a 1:100 dilution in water) were used in the second amplification reaction employing the nested primers 5'-GTTGTAGTGTGCTGCGAGCAATT-3' (SEQ ID NO: 22), an antisense-strand primer corresponding to amino acid residues 6-13 (KFAAAHYN, SEQ ID NO: 23) of the Flt4 ligand, and 5'-TCACTATAGGGAGACCCCAAGC-3' (SEQ ID NO: 24), a sense-strand primer corresponding to nucleotides 2179-2199 of the pcDNA1 vector. The sequences of these sense and antisense primers overlapped with the 3' ends of the corresponding primers used in the first PCR. "Touchdown" PCR was carried out by decreasing the annealing temperature from 72° C. to 66° C. and continuing with 18 additional cycles at 66° C. The annealing time was 1 minute and extension at each cycle was carried out at 72° C. for 2 minutes. One major product of about 220 bp and three minor products of about 270 bp, 150 bp, and 100 bp were obtained.

The amplified fragment of approximately 220 bp was cut out from the agarose gel, cloned into a pCRII vector using the TA cloning kit (Invitrogen) and sequenced. Three recombinant clones were analysed and they contained the sequence: 5'-TCACTATAGGGAGACCCCAAGCTTGGTACCGAGCTCGGATCCACTA GTAACGGCCGCCAGTGTGGTGAATTCCGAGCAACTATGACTGTA CTCTACCCAGAATATTGGAAAATGTACAAGTGTCAAGCTAAGGCAA GGAGGCTGGCAACATAACAGAGAACAGGCCAACCTCAACTCAAG GACAGAAGAGACTATAAAATTCGCTGCAGCAGCACTACAAC-3' (SEQ ID NO: 25). The beginning of the

sequence represents the pcDNA1 vector and the underlined sequence represents the amplified product of the 5'-end of the insert. The ATG codon located upstream of that sequence in the same reading frame is followed by an open reading frame containing the amplified product of the putative signal sequence and the first 13 amino acid residues of the secreted Flt4 ligand.

EXAMPLE 9

Amplification of the 3'-end of cDNA Encoding the Flt4 Ligand

Based upon the amplified 5'-sequence of the clones encoding the Flt4 ligand, two pairs of non-overlapping nested primers were designed to amplify the 3'-portion of the clones. The sense-strand primer 5'-ACAGAGAACAGGCCAAC-3' (SEQ ID NO: 26) and antisense-strand primer 5'-TCTAGCATTAGGTGACAC-3' (SEQ ID NO: 27) corresponding to nucleotides 2311-2329 of the pcDNA1 vector were used in a first "touchdown" PCR. The annealing temperature of the reaction was decreased 1° C. every two cycles from 72° C. to 52° C., at which temperature 15 additional cycles were carried out. The annealing time was 1 minute and extension at each cycle was carried out at 72° C. for 3 minutes. DNA fragments of several sizes were obtained in the first amplification. Those products were diluted 1:200 in water and reamplified in PCR using the second pair of primers: 5'-AAGAGACTATAAAATTCGCTGCAGC-3' (SEQ ID NO: 28) and 5'-CCCTCTAGATGCATGCTCGA-3' (SEQ ID NO: 29) (antisense-strand primer corresponding to nucleotides 2279-2298 of the pcDNA1 vector). Two DNA fragments were obtained, having sizes of 1350 bp and 570 bp. Those fragments were cloned into a pCRII vector and the inserts of the clones were sequenced. Both of these fragments were found to contain sequences encoding an amino acid sequence homologous to the VEGF sequence.

EXAMPLE 10

Screening the PC-3 Cell cDNA Library using the 5' PCR Fragment of Flt4 Ligand cDNA

A 219 bp 5'-terminal fragment of Flt4 ligand cDNA was amplified by PCR using the 5' PCR fragment described above and primers 5'-GTTGTAGTGTGCTGCAGCGAATTT-3' (antisense-strand primer, SEQ ID NO: 30) and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 31) (sense-primer corresponding to nucleotides 2179-2199 of the pcDNA1 vector). The amplified product was subjected to digestion with EcoRI (Boehringer Mannheim) to remove the portion of the DNA sequence amplified from the pcDNA1 vector and the resulting 153 bp fragment encoding the 5' end of the Flt4 ligand was labeled with [³²P]-dCTP using the Klenow fragment of *E. coli* DNA polymerase I (Boehringer Mannheim). That fragment was used as a probe for hybridization screening of the amplified PC-3 cell cDNA library.

Filter replicas of the library were hybridized with the radioactively labeled probe at 42° C. for 20 hours in a solution containing 50% formamide, 5xSSPE, 5xDenhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA. Filters were washed twice in 1xSSC, 0.1% SDS for 30 minutes at room temperature, then twice for 30 minutes at 65° C. and exposed overnight.

On the basis of autoradiography, 10 positive recombinant bacterial colonies hybridizing with the probe were chosen

from the library. Plasmid DNA was purified from these colonies and analysed by EcoRI and NotI digestion and agarose gel electrophoresis followed by ethidium bromide staining. The ten plasmid clones were divided into three groups on the basis of the presence of insert sizes of approximately 1.7, 1.9 and 2.1 kb, respectively. Inserts of plasmids from each group were sequenced using the T7 oligonucleotide as a primer and walking primers for subsequent sequencing reactions.

Sequence analysis showed that all clones contain the open reading frame encoding the NH₂-terminal sequence of the Flt4 ligand. Furthermore, the 2.1 and 1.9 kb clones also contained sequences encoding the signal sequence. The 5' end of the 1.7 kb clone began within the signal sequence-encoding portion. Dideoxy sequencing was continued using walking primers in the downstream direction. An 1140 nucleotide portion of the sequence of the longest clone is shown in FIGS. 9A through 9C (SEQ ID NOS: 32 and 33). As can be seen in that figure, after the putative signal sequence the open reading frame terminates in a TAA stop codon 317 amino acid residues further downstream from the signal sequence. When compared with sequences in the GenBank Database, the predicted protein product of this reading frame was found to be homologous with the predicted amino acid sequences of the PDGF/VEGF family of growth factors, as shown in FIGS. 10A and 10B.

EXAMPLE 11

Stimulation of Flt4 Autophosphorylation by the Protein Product of the Flt4 Ligand Vector

The 2.1 kb insert of the Flt4-L clone in pcDNA1 vector containing the open reading frame encoding the sequence shown in FIGS. 9A through 9C (SEQ ID NO: 32) was cut out from the vector using HindIII and NotI restriction enzymes, isolated from a preparative agarose gel and ligated to the corresponding sites in the pREP7 expression vector (Invitrogen). The pREP7 vector containing the above cloned insert was transfected into 293-EBNA cells (Invitrogen) using the calcium phosphate transfection method (Sambrook et al., Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989). About 48 hours after transfection the medium of the transfected cells was changed to DMEM medium lacking fetal calf serum and incubated for 36 h. The thus conditioned medium was then collected, centrifuged at 5000xg for 20 minutes, the supernatant was concentrated 5-fold using Centrprep 10 (Amicon) stimulate NIH3T3 cells expressing LTRFlt41, as in Example 4. The cells were lysed, immunoprecipitated using anti-Flt4 antiserum and analysed by Western blotting using anti-phosphotyrosine antibodies.

As can be seen from FIG. 11, lanes 1 and 3, the conditioned medium from two different dishes of the transfected cells stimulated Flt4 autophosphorylation in comparison with the medium from mock-transfected cells, which gave only background levels of phosphorylation of the Flt4 receptor (lane 2). When the concentrated conditioned medium was preabsorbed with 20 µl of a slur of Flt4EC domain coupled to Sepharose (see example 4), no phosphorylation was obtained (lane 4), showing that the activity responsible for Flt4 autophosphorylation was indeed the Flt4 ligand. Thus, these results demonstrate that the Flt4-L plasmid vector clone having an approximately 2.1 kb insert and containing the open reading frame shown in FIGS. 9A through 9C is expressed into a Flt4 ligand in cells transfected with the Flt4-L expression vector clone, and thus is biologically active. The sequence encoded by that open reading frame is shown in SEQ ID NO: 33. Plasmid pFLt4-L has been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852 as accession

15

number 97231. A 1997 base pair nucleotide sequence and the deduced amino acid sequence of the cDNA insert of this deposited plasmid is set forth in SEQ ID NOS: 34 and 35, respectively.

However, the predicted molecular weight of the mature protein product deduced from this reading frame is 35,724 and the Flt4 ligand from PC-3 cell cultures had an approximate molecular weight of 23 kD under reducing conditions. It is thus possible that the Flt4 mRNA may be first translated into a precursor, from which the mature ligand is derived by proteolytic cleavage. The difference in the observed molecular weight of the isolated Flt4 ligand and the deduced molecular weight of the disclosed open reading frame of the Flt4 ligand sequence may then derive from sequences in the carboxyl terminal region of the latter. Also, the Flt4 ligand may be glycosylated at two putative N-linked glycosylation sites conforming to the consensus which can be identified in the deduced Flt4 ligand amino acid sequence (N-residues underlined in FIG. 10A).

The carboxyl terminal amino acid sequences, which increase the predicted molecular weight of the Flt4 ligand subunit in comparison with other ligands of this family show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring protein 3 (BRP3) sequence (Dignam and Case, *Gene* 88, 133-140, 1990). Such a sequence may encode an independently folded domain present in a Flt4 ligand precursor and it may be involved, for example, in the regulation of secretion, solubility, stability, cell surface localization or activity of the Flt4 ligand. Interestingly, at least one cysteine motif of the BRP3 type is also found in the VEGF carboxy terminal amino acid sequences.

Thus, the Flt4 mRNA may be first translated into a precursor from the mRNA corresponding to the Flt4-L clone, from which the mature ligand is derived by proteolytic cleavage. To define the mature Flt4 ligand product one first expresses the cDNA clone, which is deposited in the pcDNA1 expression vector, in cells, such as COS cells and use antibodies generated against Flt4-L-encoded peptides, such as amino terminal 23 amino acid peptide or bacterial Flt4 fusion proteins, such as a GST-fusion protein, to raise antibodies against the VEGF-homologous domain of Flt4 ligand. One then follows the biosynthesis and processing of the Flt4 ligand in the transfected cells by pulse-chase analysis using radioactive cysteine for labelling of the cells, immunoprecipitation and gel electrophoresis. Using antibodies against the two domains of the product of the Flt4-L clone material for radioactive or nonradioactive aminoterminal sequence analysis is isolated. The determination of the NH2-terminal sequence of the carboxyl terminal fragment allows for identification of the proteolytic processing site. This is confirmed by site-directed mutagenesis of the amino acid residues adjacent to the cleavage site, which would prevent the cleavage.

16

On the other hand, the Flt4 ligand is characterized by progressive 3' deletions in the 3' coding sequences of the Flt4 ligand precursor clone, resulting in carboxy-terminal truncations of its protein product. The activities of such truncated forms are assayed by, for example, studying Flt4 autophosphorylation induced by the truncated proteins when applied to cultures of cells, such as NIH3T3 cells expressing LTRFlt4. By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin et al., *Growth Factors* 8, 245-252, 1993) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues.

On the other hand, the difference between the molecular weights of the purified ligand and the open reading frame of the Flt4 precursor clone may be due to the fact that the soluble ligand was produced from an alternatively spliced mRNA which would also be present in the PC-3 cells, from which the isolated ligand was derived. To isolate such alternative cDNA clones one uses cDNA fragments of the deposited clone and PCR primers made according to the sequence provided as well as techniques standard in the art to isolate or amplify alternative cDNAs from the PC-3 cell cDNA library. One may also amplify using reverse transcription (RT)-PCR directly from the PC-3 mRNA using the primers provided in the sequence of the Flt4-L clone. Alternative cDNAs can be sequenced from the resulting cDNA clones. One can also isolate genomic clones corresponding to the Flt4-L transcript from a human genomic DNA library using methods standard in the art and to sequence such clones or their subcloned fragments to reveal the corresponding exons. Alternative exons can then be identified by a number of methods standard in the art, such as heteroduplex analysis of cDNA and genomic DNA and they can subsequently be characterized.

EXAMPLE 12

Expression of the Flt4-L Gene

Expression of transcripts corresponding to the Flt4 ligand was analysed by hybridization of Northern blots containing isolated polyA+RNA from HT-1080 and PC-3 human tumor cell lines. The probe was the radioactively labelled insert of the 2.1 kb cDNA clone (specific activity 10^8 - 10^9 cpm/mg of DNA). The blot was hybridized overnight at 42° C. or using 50% formamide, 5xSSPE buffer, 2% SDS, 10xDenhardt's solution, 100 mg/ml salmon sperm DNA and 1×10^6 cpm of the labelled probe/ml. The blot was washed at room temperature for 2x30 minutes in 2xSSC containing 0.05% SDS and then for 2x20 min at 52° C. in 0.1xSSC containing 0.1% SDS. The blot was then exposed at -70° C. for three days using intensifying screens and Kodak XAR film. Both cell lines expressed an Flt4 ligand mRNA of about 2.3 kb, as well as VEGF and VEGF-B mRNA:s (FIG. 12).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(111) NUMBER OF SEQUENCES: 35

(2) INFORMATION FOR SEQ ID NO: 1:

(4) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TGTCCTCGCT GTCCCTGCT 20

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 70 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ACATGCATGC CACCATGCAG CCGGGGCCCG CGCTGTGCT GCGACTGTGG CTCTGCTGG 60
 GACTCTGGA 70

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ACATGCATGC CCGCGCGTC ATCC 24

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CGGAATCCC CATGACCCA AC 22

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CAATGATGG ATCTACTCTG AATCCCTTT CTT 33

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 base pairs
 (B) TYPE: nucleic acid

-continued

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATTAGGTGA CACTATA 17

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT 34

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Pro	Met	Thr	Pro	Thr	Thr	Tyr	Lys	Gly	Ser	Val	Asp	Asn	Gln	Thr	As
1				5					10				15		
Ser	Gly	Met	Val	Leu	Ala	Ser	Glu	Glu	Phe	Glu	Gln	Ile	Glu	Ser	Ar
			20				25					30			
His	Arg	Gln	Glu	Ser	Gly	Phe	Arg								
			35				40								

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CTGGAGTCGA CTGGCGGAC T 21

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CGCGGATCCC TAGTGATGAT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCTTC 60

(2) INFORMATION FOR SEQ ID NO: 11:

-continued

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 34 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:
- CCCAAGCTTG GATCCAGTGC GCTACTCCAT GACC 34
- (2) INFORMATION FOR SEQ ID NO: 12:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
- GTTGCCTGTG ATGTGCACCA 20
- (2) INFORMATION FOR SEQ ID NO: 13:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
- Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Il
 1 5 10 15
 Leu Lys
- (2) INFORMATION FOR SEQ ID NO: 14:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:
- GCAGAGAGA CAAATAA 17
- (2) INFORMATION FOR SEQ ID NO: 15:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:
- Glu Glu Thr Ile Lys
 1 5
- (2) INFORMATION FOR SEQ ID NO: 16:

-continued

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GCAATTNARD ATTTCTGT 18

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Thr Glu Ile Leu Lys
 1 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

ATTGCTGTGCA GCACACTACA AC 22

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TCACTGTGTGT AGTGTGCTG 19

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Ala Ala His Tyr Asn Thr Glu
 1 7

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TAAATCCGACT CACTATAGGG 20

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GTCTAGTGT GCTGCACGA ATTT 24

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Lys Phe Ala Ala His Tyr Asn
1 5

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

TCACTATAGG GAGACCCAG C 21

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 219 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

TCACTATAGG GAGACCCAG CTTCGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT 60

GTCTGGGAT TCAGCAACT CATGACTGTA CTCTACCCAG AATATTGGAA AATGTACAA 120

TGTACCTAA GTCACACAG CTGCAACAT AACGAGAAC AGGCTAACCT CAACTCAG 180

ACCAACAGA CTATAAATT CCTGACCA CACTACAA 219

-continued

(2) INFORMATION FOR SEQ ID NO: 26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

ACAGAGACCA GCCCAACC

18

(2) INFORMATION FOR SEQ ID NO: 27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TCTAGCATT AGGTGACAC

19

(2) INFORMATION FOR SEQ ID NO: 28:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

AAGAGACTAT AAAATTGCT GCAGC

25

(2) INFORMATION FOR SEQ ID NO: 29:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CCCTCTAGT GCATGCTGA

20

(2) INFORMATION FOR SEQ ID NO: 30:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GTGTAGTGT GCTGCAAGA ATTT

24

(2) INFORMATION FOR SEQ ID NO: 31:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs

-continued

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

TCACATATAGG GAGACCCAAG C 21

(c) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1140 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 37..1089

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAATCTC ATG ACT GTA CTC TAC CCA 54
Met Thr Val Leu Tyr Pro
1 5

GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA 102
Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln
10 15 20

CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA 150
His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile
25 30 35

AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT 198
Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp
40 45 50

AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CCG GAG GTG TOT ATA GAT 246
Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp
55 60 65 70

GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA 294
Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro
75 80 85

TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG 342
Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu
90 95 100

CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA 390
Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu
105 110 115

ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT 438
Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe
120 125 130

GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA 486
Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg
135 140 145 150

CAA GTT CAT TCC ATT ATT AGA GGT TCC CTG CCA GCA ACA CTA CCA CAG 534
Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln
155 160 165

TUT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT 582
Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn
170 175 180

CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT 630
His Ile Cys Arg Cys Leu Ala Gln Asp Phe Met Phe Ser Ser Asp
185 190 195

-continued

GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC	678
Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn	
200 205 210	
AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT	726
Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu	
215 220 225 230	
CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC	774
Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys	
235 240 245	
CAG TGT GTC TGT AAA AAC AAA CTC TTC CCC AGC CAA TGT GCG GCC AAC	822
Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn	
250 255 260	
CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC	870
Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys	
265 270 275	
CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA	918
Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr	
280 285 290	
GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA	966
Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln	
295 300 305 310	
ACA TGC AGC TGT TAC AGA CCG CCA TGT ACG AAC CGC CAG AAG GCT TGT	1014
Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys	
315 320 325	
GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA	1062
Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser	
330 335 340	
TAT TGG AAA AGA CCA CAA ATG AGC TAAGATTGTA CTGTTTTCGA GTTCATCGA	1116
Tyr Trp Lys Arg Pro Gln Met Ser	
345 350	
TTTCTATTAT GGAAAACTGT GTTG	1140

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 150 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu
 1 5 10 15

Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser
 20 25 30

Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu
 35 40 45

Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro
 50 55 60

Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn
 65 70 75 80

Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys
 85 90 95

Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu
 100 105 110

Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys
 115 120 125

Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser

-continued

120	135	140
Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu		
145	150	155
Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr		
	165	170
Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp		
	180	185
Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His		
	195	200
Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys		
	210	215
Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu		
	225	230
Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro		
	245	250
Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys		
	260	265
Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys		
	275	280
Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly		
	290	295
Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr		
	305	310
Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val		
	325	330
Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser		
	340	345

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1997 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CCCGCCCCGC CTCTCCAAAG AGCTACACCG ACCGGGACCG CGCGGGCTTC CTCCTTCGCC	60
CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCCGGTTT CCGTGTAGG	120
TTTACCTGA CACCCGCGGC CTTTCGCCGG CACTGGCTGG GAGGGCGCCC TCAGAGTT	180
GGACCGCGGA GCCCCGAGCC CGCTCCCGCC GCTCCGGCT CCGCCAGGGG GCGTGGCC	240
AGAGATATTT (GGGGGGGG) ATATATATAT GCTTGTGTTC TCCAGATATTC GCTTGTGTTC	300
ATATATATAT (GGGGGGGG) ATATATATAT GCTTGTGTTC TCCAGATATTC GCTTGTGTTC	360
TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCG GCT GCG CTG	405
Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Ala Ala Ala Leu	
5 10 15	
CTT CCG GGT CTT TTT GAG GCG CTT GCG GCG GCG GCG GCG TTC GAG TTC	450
Leu Phe Gly Phe Arg Gln Ala Phe Ala Ala Ala Ala Ala Phe Gln Met	
20 25 30	

-continued

GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GCG GAG CCC ACG GCT Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala 35 40 45 50	501
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CCG TCT GTG TCC AGT GTA Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val 55 60 65	549
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys 70 75 80	597
TGT CAG CTA AGG AAA GGA GCG TGG CAA CAT AAC AGA GAA CAG GCC AAC Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn 85 90 95	645
CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr 100 105 110	693
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln 115 120 125 130	741
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val 135 140 145	789
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys 150 155 160	837
GCG GGT TGC TGC AAT AGT GAG GCG CTG CAG TGC ATG AAC ACC AGC ACG Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr 165 170 175	885
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln 180 185 190	933
GCG CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg 195 200 205 210	981
TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg 215 220 225	1029
GCT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr 230 235 240	1077
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala 245 250 255	1125
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp 260 265 270	1173
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG CAG CTG GAT GAA GAG ACC Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr 275 280 285 290	1221
TTT CAG TTT GTC TTT ATA GTG GAG CTT CCG CTT GTC AAG TGT GGA CCC Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Met Cys Gly Pro 295 300 305	1269
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys 310 315 320	1317
CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr 325 330 335	1365
TTT CAG TTT ATA TTT AAA ATA ACT TGC CCC ATA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn 340 345 350	1411

-continued

340	345	350	
CCT GGA AAA TGT GGC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG			1461
Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu			
355	360	365	370
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG			1509
Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg			
375	380	385	
CCA TGT ACG AAC GGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT			1557
Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser			
390	395	400	
GAA GAA GTG TGT GGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG			1605
Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met			
405	410	415	
AGC TAAGATTGTA CTGTTTCCA GTTCATCGAT TTCTATTAT GGAAAACTGT			1658
Ser			
GTGCCACAG TAGAAGTGTG TGTGAACAGA GAGACCCCTG TGGGTCCATG CTAACAAA			1718
CAAAAGTCTG TCTTTACTGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGCTCA			1778
TGGAAAGGCC CTCTGTAAA GACTGGTTT CTGCCAATGA CCAAAACAGCC AAGATTTT			1838
TCTGTGATT TCTTTAAAG AATGACTATA TAATTATTT CCACTAAAAA TATTGTTT			1898
GCATTCATT TTATAACAAC AACAATTGGT AAAACTCACT GTGATCAATA TTTTATA			1958
ATGCAAAATA TTTTAAAT AAAATGAAAA TTGTATTAT			1997

(2) INFORMATION FOR SEQ ID NO: 35:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 419 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
 1 5 10 15

Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe
 20 25 30

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
 35 40 45

Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
 50 55 60

Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
 65 70 75 80

Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
 85 90 95

Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
 100 105 110

His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
 115 120 125

Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asn Val Gly Lys Glu Phe
 130 135 140

Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
 145 150 155 160

Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
 165 170 175

Met Thr Met Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu

-continued

180								185								190							
Ser	Gln	Gly	Pro	Lys	Pro	Val	Thr	Ile	Ser	Phe	Ala	Asn	His	Thr	Ser								
		195					200						205										
Cys	Arg	Cys	Met	Ser	Lys	Leu	Asp	Val	Tyr	Arg	Gln	Val	His	Ser	Ile								
	210					215						220											
Ile	Arg	Arg	Ser	Leu	Pro	Ala	Thr	Leu	Pro	Gln	Cys	Gln	Ala	Ala	Asn								
	225				230						235				240								
Lys	Thr	Cys	Pro	Thr	Asn	Tyr	Met	Trp	Asn	Asn	His	Ile	Cys	Arg	Cys								
			245						250					255									
Leu	Ala	Gln	Glu	Asp	Phe	Met	Phe	Ser	Ser	Asp	Ala	Gly	Asp	Asp	Ser								
			260					265					270										
Thr	Asp	Gly	Phe	His	Asp	Ile	Cys	Gly	Pro	Asn	Lys	Glu	Leu	Asp	Glu								
		275					280					285											
Glu	Thr	Cys	Gln	Cys	Val	Cys	Arg	Ala	Gly	Leu	Arg	Pro	Ala	Ser	Cys								
		290					295					300											
Gly	Pro	His	Lys	Glu	Leu	Asp	Arg	Asn	Ser	Cys	Gln	Cys	Val	Cys	Lys								
	305				310						315				320								
Asn	Lys	Leu	Phe	Pro	Ser	Gln	Cys	Gly	Ala	Asn	Arg	Glu	Phe	Asp	Glu								
			325						330					335									
Asn	Thr	Cys	Gln	Cys	Val	Cys	Lys	Arg	Thr	Cys	Pro	Arg	Asn	Gln	Pro								
			340					345					350										
Leu	Asn	Pro	Gly	Lys	Cys	Ala	Cys	Glu	Cys	Thr	Glu	Ser	Pro	Gln	Lys								
			355				360					365											
Cys	Leu	Leu	Lys	Gly	Lys	Lys	Phe	His	His	Gln	Thr	Cys	Ser	Cys	Tyr								
		370					375					380											
Arg	Arg	Pro	Cys	Thr	Asn	Arg	Gln	Lys	Ala	Cys	Glu	Pro	Gly	Phe	Ser								
	385				390					395					400								
Tyr	Ser	Glu	Glu	Val	Cys	Arg	Cys	Val	Pro	Ser	Tyr	Trp	Lys	Arg	Pro								
				405					410					415									
Gln	Met	Ser																					

What is claimed is:

1. A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of amino acids 1-180 of SEQ ID NO: 33 effective to permit such binding, said polypeptide lacking all of amino acids of SEQ ID NO: 33 beyond position 180.
2. A pharmaceutical composition comprising a polypeptide according to claim 1 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.
3. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim 2.
4. A polypeptide according to claim 1 further comprising a detectable label.
5. A purified and isolated polypeptide according to claim 1 that binds Flt4 and stimulates Flt4 phosphorylation in mammalian cells expressing Flt4.
6. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim 1.
7. A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase (Flt4), wherein the polypeptide comprises a portion of SEQ ID NO: 33 effective to permit such binding.

and wherein the polypeptide has an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

8. A pharmaceutical composition comprising a polypeptide according to claim 7 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

9. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim 8.

10. A purified and isolated polypeptide according to claim 7 that binds Flt4 and stimulates Flt4 phosphorylation in mammalian cells expressing Flt4.

11. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim 10.

12. A purified and isolated polypeptide according to claim 10, said polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13.

13. A polypeptide according to claim 7 further comprising a detectable label.

14. A polypeptide according to claim 7 wherein said portion of SEQ ID NO: 33 effective to permit such binding is a continuous portion of SEQ ID NO: 33 within amino acids 1-180 of SEQ ID NO: 33.

15. A polypeptide according to claim 7 wherein the amino terminus of said portion effective to permit such binding corresponds with position 34 of SEQ ID NO: 33.

16. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim 7.

17. A purified and isolated polypeptide comprising a human polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, wherein amino terminal amino acids 2 through 18 of said human polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

18. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim 17.

19. A purified and isolated polypeptide according to claim 17 that binds Flt4 and stimulates Flt4 phosphorylation in mammalian cells expressing Flt4.

20. A pharmaceutical composition comprising a polypeptide according to claim 17 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

21. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim 20.

22. A polypeptide according to claim 17 further comprising a detectable label.

23. A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, wherein said polypeptide has an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions and is purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

24. A polypeptide according to claim 23 which is capable of stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

25. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim 24.

26. A polypeptide according to claim 23 further comprising a detectable label.

27. A pharmaceutical composition comprising a polypeptide according to claim 23 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

28. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim 27.

29. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim 23.

* * * * *

34013
5/4
12
Class
Subcla
ISSUE CLASSIFICATION



6221839



PCT NUMBER 08/08/10133	PATENT DATE APR 24 2001	PATENT NUMBER 6221839
----------------------------------	----------------------------	--------------------------

SERIAL NUMBER 08/510,133	FILING DATE 08/01/95	CLASS 536 514	SUBCLASS 12	GROUP ART UNIT 1814 1814	EXAMINER 60A
-----------------------------	-------------------------	---------------------	----------------	--------------------------------	-----------------

APPLICANTS KARI ALITALO, ESPOO, FINLAND; VLADIMIR JOUKOV, HELSINKI, FINLAND. *Sisva*

CONTINUING DATA
VERIFIED

FOREIGN/PCT APPLICATIONS
VERIFIED

Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED →	STATE OR COUNTRY FIN	SHEETS DRWGS. 12	TOTAL CLAIMS 12	INDEP. CLAIMS 3	FILING FEE RECEIVED \$860.00	ATTORNEY'S DOCKET NO. 28113/328
---	--	---------------	-------------------------	---------------------	--------------------	--------------------	---------------------------------	------------------------------------

Verified and Acknowledged Examiner's Initials *BRL*

ADDRESS MARSHALL O'TOOLE GERSTEIN MURRAY *David A. Gerst*
AND BORUN *Marshall O'Toole Gerstein Murray & Borun*
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402 *6300 Sears Tower*
233 South Wacker Drive
Chicago, Illinois 60606-6402

TITLE RECEPTOR LIGAND
Fit 4 Ligand and Methods of Use

U.S. DEPT. of COMMERCE • Patent and Trademark Office • PCT-436L (rev. 7-)

PARTS OF APPLICATION FILED SEPARATELY		A.G. Hannell Applications Examiner	
NOTICE OF ALLOWANCE MAILED		CLAIMS ALLOWED	
10/3/00		Total Claims 29	Print Claim 1
ISSUE FEE Amount Due 1240.00 Date Paid 1-5-01		DRAWING Sheets Drwg. 76-12 Figs. Drwg. 19-12 Print Fig. none	
Label Area		CHRISTINE SAOUD PATENT EXAMINER <i>Christine Saoud</i> Primary Examiner	
PREPARED FOR ISSUE		ISSUE BATCH NUMBER 103	
WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 181 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.			

Form PTO-436A
(Rev. 8/92)

ISSUE FEE IN FILE
Formal Drawings/ sheets/set
(FACE)

08/510133

PATENT APPLICATION



08510133

APPROVED FOR LICENSE

INITIALS SEP 1 9 53

Date
Entered
or
Counted

CONTENTS

Date
Received
or
Mailed

RECEIVED

FEB 20 1996

GHUUR 1000

1. Application	12 sheets	papers.	
2. Statement	1-821		8-1-95
3. Laws listing	(9)		10-4-95
4. Lile, MSO			12-21-95
5. Statement	1-821		11-7-95
6. TDS			5-29-96
7. Restriction	30 Days		7-26-96
8. Rej. 1			7-26-96
9. Statement			8-12-96
10. Rej. 3 mos.			9-10-96
11. Fact sheet			5-14-96
12. DS			2-13-97
13. Time			2-13-97
14. Time			2-13-97
15. Time			04-11-97
16. Time			4/5/97
17. Time			3/3/97
18. Time			6/11/97
19. Time			6/25/97
20. Time			12/29/97
21. Time			03 APR 1998
22. Time			02/26/98
23. Time			7-26-00
24. Time			6-28-00
25. Time			7/26/00
26. Time			8/5/00
27. Time			10-3-00
28. Time			11/2/00
29. Time			
30. Time			
31. Time			
32. Time			

(FRONT)

SEARCHED			
Class	Sub.	Date	Exmr.
530	399	8/28	BKL
514	2	1	1
	21		
updated		4/3/97	BKL
search update		4/10/00	CL
search update		9/27/00	CL

INTERFERENCE SEARCHED			
Class	Sub.	Date	Exmr.
530	399	9/27/00	CL
514	2	1	1
	12	1	1

SEARCH NOTES		
	Date	Exmr.
USPAT search enclosed	8/22/96	BKL
MEDLINE, WPIPS, SCI- SEARCH search enclosed	8/22/96	BKL
EMBASE, CAPLUS, SCISEARCH search enclosed.	8/23/96	BKL
Protein sequence database search - see enclosure. See Genesys, Swissprot, pif & excerpts.	8/13/96	BKL
USPAT search updated	4/3/97	BKL
Seq ID NO: 13, 33,	10/18/99	CL
AA 2-180613.	4/10/00	CL
search update		
Discussed case w/ Mentor center (clm 45)	9/8/00	CL
update	9/27/00	CL

(RIGHT OUTSIDE)

POSITION	ID NO.	DATE
CLASSIFIER		
EXAMINER	N/A	9-19-95
TYPIST	21	11-14
VERIFIER		2/1/96
CORPS CORR.		
SPEC. HAND	N/A	1-29
FILE MAINT.	823	11/1/95
DRAFTING		

INDEX OF CLAIMS


Claim	Final	Original	Date
1	29		
2	30		
3	31		
4	32		
5	33		
6	34		
7	35		
8	36		
9	37		
10	38		
11	39		
12	40		
13	41		
14	42		
15	43		
16	44		
17	45		
18	46		
19	47		
20	48		
21	49		
22	50		

Claim	Final	Original	Date
23	51		
24	52		
25	53		
26	54		
27	55		
28	56		
29	57		
30	58		
31	59		
32	60		
33	61		
34	62		
35	63		
36	64		
37	65		
38	66		
39	67		
40	68		
41	69		
42	70		
43	71		
44	72		
45	73		
46	74		
47	75		
48	76		
49	77		
50	78		
51	79		
52	80		
53	81		
54	82		
55	83		
56	84		
57	85		
58	86		
59	87		
60	88		
61	89		
62	90		
63	91		
64	92		
65	93		
66	94		
67	95		
68	96		
69	97		
70	98		
71	99		
72	100		

SYMBOLS

✓ Rejected
 = Allowed
 (Through numeral) Canceled
 + Restricted
 N Non-elected
 I Interference
 A Appeal
 O Objected

(LEFT INSIDE)

BAR CODE LABEL 		U.S. PATENT APPLICATION			
SERIAL NUMBER 08/510,133		FILING DATE 08/01/95		CLASS 536	GROUP ART UNIT 1814
APPLICANT	KARI ALITALO, ESPOO, FINLAND; VLADIMIR JOUKOV, HELSINKI, FINLAND.				
	CONTINUING DATA*** VERIFIED _____				
	FOREIGN/PCT APPLICATIONS*** VERIFIED _____				
STATE OR COUNTRY FIX	SHEETS DRAWING 12	TOTAL CLAIMS 12	INDEPENDENT CLAIMS 3	FILING FEE RECEIVED \$860.00	ATTORNEY DOCKET NO. 28113/32863
ADDRESS	MARSHALL O'TOOLE GERSTEIN MURRAY AND BORUN 6300 SEARS TOWER 233 SOUTH WACKER DRIVE CHICAGO IL 60606-6402				
TITLE	RECEPTOR LIGAND				
This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application which is identified above. By authority of the COMMISSIONER OF PATENTS AND TRADEMARKS					
Date		Certifying Officer			

PATENT APPLICATION SERIAL NO. **08/510133**

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

270 BA 08/30/95 08510133

1 101

730.00 OK 28113/32863



730-101

Box
SEQUENCE
6/510133

- 1 -

RECEPTOR LIGAND

FIELD OF THE INVENTION

The present invention generally relates to the field of genetic engineering and more particularly to growth factors for endothelial cells and growth factor genes.

BACKGROUND OF THE INVENTION

Developmental growth, the remodelling and regeneration of adult tissues as well as solid tumor growth, can only occur when accompanied by blood vessel formation. Angioblasts and hematopoietic precursor cells differentiate from the mesoderm and form the blood islands of the yolk sac and the primary vascular system of the embryo. The development of blood vessels from these early (*in situ*) differentiating endothelial cells is termed vasculogenesis. Major embryonic blood vessels are believed to arise via vasculogenesis, whereas the formation of the rest of the vascular tree is thought to occur as a result of vascular sprouting from pre-existing vessels, a process called angiogenesis, Risau, *et al.*, *Devel. Biol.*, 125: 441-450 (1988).

Endothelial cells give rise to several types of functionally and morphologically distinct vessels. When organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases. Upon angiogenic stimulation, endothelial cells may re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their tissue environment. Endothelial cells undergoing angiogenesis degrade the underlying basement membrane and migrate, forming capillary sprouts that project into the perivascular stroma. Ausprunk, *et al.*, *Microvasc. Rev.*, 14: 51-65 (1977). Angiogenesis during tissue development and regeneration depends on the tightly controlled processes of endothelial cell proliferation,

migration, differentiation and survival. Dysfunction of the endothelial cell regulatory system is a key feature of many diseases. Most importantly, tumor growth and metastasis have been shown to be angiogenesis dependent. Folkman, *et al.*, *J. Biol. Chem.*, 267:10931-10934 (1992).

5 Key signals regulating cell growth and differentiation are mediated by polypeptide growth factors and their transmembrane receptors, many of which are tyrosine kinases. Autophosphorylated peptides within the tyrosine kinase insert and carboxyl-terminal sequences of activated
10 receptors are commonly recognized by kinase substrates involved in signal transduction for the readjustment of gene expression in responding cells. Several families of receptor tyrosine kinases have been characterized. Van der Geer, *et al.*, *Ann. Rev. Cell Biol.*, 10:251-337 (1994). The major growth factors and receptors transducing angiogenic stimuli are schematically shown in Figure 1.

15 Fibroblast growth factors are also known to be involved in the regulation of angiogenesis. They have been shown to be mitogenic and chemotactic for cultured endothelial cells. Fibroblast growth factors also stimulate the production of proteases, such as collagenases and plasminogen activators, and induce tube formation by endothelial cells. Saksela, *et al.*,
20 *Ann. Rev. Cell Biol.*, 4:93-126 (1988). There are two general classes of fibroblast growth factors, FGF-1 and FGF-2, both of which lack conventional signal peptides. Both types have an affinity for heparin and FGF-2 is bound to heparin sulfate proteoglycans in the subendothelial extracellular matrix from which it may be released after injury. Heparin
25 potentiates the stimulation of endothelial cell proliferation by angiogenic FGFs, both by protecting against denaturation and degradation and dispersing the FGFs. Cultured endothelial cells express the FGF-1 receptor but no significant levels of other high-affinity fibroblast growth factor receptors.

30 Among other ligands for receptor tyrosine kinases, the

platelet derived growth factor, PDGF-BB, has been shown to be weakly angiogenic in the chick chorioallantoic membrane. Risau, *et al.*, *Growth Factors*, 7:261-266 (1992). Transforming growth factor α (TGF α) is an angiogenic factor secreted by several tumor cell types and by macrophages.

- 5 Hepatocyte growth factor (HGF), the ligand of the *c-met* proto-oncogene-encoded receptor, is also strongly angiogenic.

- Recent evidence shows that there are endothelial cell specific growth factors and receptors that may be primarily responsible for the stimulation of endothelial cell growth, differentiation and certain
10 differentiated functions. The best studied of these is vascular endothelial growth factor (VEGF), a member of the PDGF family. Vascular endothelial growth factor is a dimeric glycoprotein of disulfide-linked 23 kDa subunits, discovered because of its mitogenic activity toward
15 endothelial cells and its ability to induce vessel permeability (hence its alternative name vascular permeability factor). Other reported effects of VEGF include the mobilization of intracellular calcium, the induction of plasminogen activator and plasminogen activator inhibitor-1 synthesis, stimulation of hexose transport in endothelial cells, and promotion of
20 monocyte migration *in vitro*. Four VEGF isoforms encoded by distinct mRNA splice variants appear to be equally capable of stimulating mitogenesis in endothelial cells. However, each has a different affinity for cell surface proteoglycans, which behave as low affinity receptors for VEGF. The 121 and 165 amino acid isoforms of VEGF are secreted in a soluble form, whereas the isoforms of 189 and 206 amino acid residues
25 remain cell surface associated and have a strong affinity for heparin.

- The pattern of VEGF expression suggests its involvement in the development and maintenance of the normal vascular system and in tumor angiogenesis. During murine development, the entire 7.5 day post-coital (p.c.) endoderm expresses VEGF and the ventricular neuroectoderm
30 produces VEGF at the capillary ingrowth stage, Breier, *et al.*, *Development*,

114:521-523 (1992). On day two of quail development, the vascularized area of the yolk sac as well as the whole embryo show expression of VEGF. In addition, epithelial cells next to fenestrated endothelia in adult mice show persistent VEGF expression, suggesting a role in the maintenance of this specific endothelial phenotype and function.

Two high affinity receptors for VEGF have been characterized. These are VEGFR-1/Flt-1 (fms-like tyrosine kinase-1) and VEGFR-2/Kdr/Flk-1 (kinase insert domain containing receptor/fetal liver kinase-1). Those receptors are classified in the PDGF-receptor family, but they have seven rather than five immunoglobulin-like loops in their extracellular domain and they possess a longer kinase insert than normally observed in this family. The expression of VEGF receptors occurs mainly in vascular endothelial cells, although some may be present on monocytes and melanoma cells. Only endothelial cells have been reported to proliferate in response to VEGF and endothelial cells from different sources show different responses. Thus, the signals mediated through VEGFR-1 and VEGFR-2 appear to be cell type specific.

The Flt4 receptor tyrosine kinase is closely related in structure to the products of the VEGFR-1 and VEGFR-2 genes. Despite this similarity, the mature form of Flt4 differs from the VEGF receptors in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola *et al.*, *Cancer Res.*, 52:5738-5743 (1992). The 4.5 and 5.8 kb Flt-4 mRNAs encode polypeptides which differ in their C-termini due to the use of alternative 3' exons. The VEGFs do not show specific binding to Flt4 or induce its autophosphorylation.

Expression of Flt4 appears to be more restricted than expression of VEGFR-1 or VEGFR-2. The expression of Flt4 first becomes detectable by *in situ* hybridization in the angioblasts of head mesenchyme, the cardinal vein, and extraembryonically in the allantois of 8.5 day p.c. mouse embryos. In 12.5 day p.c. embryos the Flt-4 signal is

observed in developing venous and presumptive lymphatic endothelia, but arterial endothelia appear negative. During later stages of development, Flt4 mRNA becomes restricted to developing lymphatic vessels. Only the lymphatic endothelia and some high endothelial venules express Flt4
5 mRNA in adult human tissues and increased expression occurs in lymphatic sinuses in metastatic lymph nodes and in lymphangioma. These results support the theory of the venous origin of lymphatic vessels.

SUMMARY OF THE INVENTION

The present invention provides a ligand for the Flt4 receptor
10 tyrosine kinase. In a preferred embodiment, the ligand comprises a fragment of the amino acid sequence shown in SEQ ID NO: 33 which specifically binds to the Flt4 receptor tyrosine kinase.

The present invention also provides a precursor of an Flt4 ligand, wherein the precursor comprises the amino acid sequence shown in
15 SEQ ID NO: 33. The precursor is proteolytically cleaved upon expression to produce an approximately 23 kD peptide which is the Flt4 ligand. In a preferred embodiment of the invention, an Flt4 ligand is provided which is the cleavage product of the precursor peptide shown in SEQ ID NO: 33 and which has a molecular weight of approximately 23 kD under reducing
20 conditions. The Flt4 ligand comprises approximately the first 180 amino acids shown in SEQ ID NO: 33.

Also in a preferred embodiment, nucleic acids encoding an Flt4 ligand precursor are presented. Due to the degeneracy of the genetic code, numerous such coding sequences are possible, each having in
25 common the coding of the amino acid sequence shown in SEQ ID NO: 33, or portions thereof. Ligand precursors according to the invention, when expressed in an appropriate host cell, produce, via cleavage, a peptide which binds specifically to the Flt4 receptor tyrosine kinase. The nucleotide sequence encoding the Flt4 ligand is within the nucleotide

sequence shown in SEQ ID NO: 32.

The present invention also provides a cell line which produces an Flt4 ligand. The ligand may be purified and isolated directly from the cell culture medium. Also provided are vectors comprising DNA
5 encoding the Flt4 ligand and host cells comprising the vectors. Vectors are capable of expressing the Flt4 ligand under the control of appropriate promoters and other control sequences.

Ligands according to the invention may be labeled with a detectable label and used to identify their corresponding receptors *in situ*.
10 Antibodies, both monoclonal and polyclonal, may be made against a ligand of the invention according to standard techniques in the art. Such antibodies may be used in diagnostic applications to monitor angiogenesis, vascularization, lymphatic vessels and their disease states, wound healing, or certain hematopoietic or leukemia cells or they may be used to block or
15 activate the Flt4 receptor. Labeled Flt4 ligand and anti-Flt4 ligand antibodies may be used as imaging agents in the detection of lymphatic vessels, high endothelial venules, and Flt4 receptors expressed in histochemical tissue sections. The ligand or antibody may be covalently or non-covalently coupled to a suitable supermagnetic, paramagnetic, electron
20 dense, echogenic, or radioactive agent for imaging. Other, non-radioactive labels, such as biotin and avidin may also be used.

The present invention also provides diagnostic and clinical applications for claimed ligands. In a preferred embodiment, Flt4 ligands or precursors of the invention are used to accelerate angiogenesis *e.g.*
25 during wound healing or to promote the endothelial functions of lymphatic vessels. Ligands may be applied in any suitable manner using an appropriate pharmaceutically-acceptable vehicle. Ligands may also be used to quantify future metastatic risk by assaying biopsy material for the presence of active receptors or ligands in a binding assay or kit using
30 detectably-labeled ligand. An Flt4 ligand according to the invention may

also be used to promote re-growth or permeability of lymphatic vessels in, for example, organ transplant patients. Ligands according to the invention may also be used to treat or prevent inflammation, edema, aplasia of the lymphatic vessels, lymphatic obstruction, elephantiasis, and Milroy's disease. Finally, Flt4 ligands may be used to stimulate lymphocyte production and maturation and to promote or inhibit trafficking of leukocytes between tissues and lymphatic vessels or to affect migration in and out of the thymus.

Inhibitors of the Flt4 ligand may be used to control endothelial cell proliferation and lymphangiomas. For example, such inhibitors may be used to arrest metastatic growth or spread or to control other aspects of endothelial cell expression and growth. Inhibitors include antibodies, antisense oligonucleotides, and peptides which block the Flt4 receptor.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram showing major endothelial cell receptor tyrosine kinases and growth factors involved in vasculogenesis and angiogenesis.

Figure 2 shows schematically the construction of the pLTRFlt4l expression vector

Figure 3 shows schematically the construction of the baculovirus vector encoding a secreted soluble Flt4EC domain

Figure 4 shows results of stimulation of Flt4 autophosphorylation by conditioned medium from PC-3 cell cultures.

Figure 5 shows that the tyrosyl phosphorylated polypeptide of Flt4-transfected cells stimulated with PC-3 conditioned medium is the 125 kD Flt4 polypeptide.

Figure 6 shows Western analysis of the Flt4 ligand activity isolated from PC-3 conditioned medium.

Figure 7 shows results of gel electrophoresis of fractions from the Western analysis of Flt4 ligand isolated from PC-3 conditioned medium.

Figure 8 shows results of Western analysis of Flt4 autophosphorylation induced by either the Flt4 ligand, VEGF, or PlGF.

Figure 9 shows the nucleotide and deduced amino acid sequence of the coding portion of Flt4 ligand cDNA.

Figure 10 shows a comparison of the deduced amino acid sequences of PDGF-A, -B, two PlGF isoforms, four VEGF isoforms and Flt4 ligand.

Figure 11 shows the stimulation of autophosphorylation of the Flt4 receptor by conditioned medium from cells transfected with the Flt4-L expression vector

Figure 12 shows Northern blotting analysis of Flt4-L mRNA in tumor cell lines.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to novel growth factors which are ligands for the Flt4 receptor tyrosine kinase. Claimed ligands are members of a family of platelet-derived growth factors/vascular endothelial growth factors which promote mitosis and proliferation of vascular endothelial cells and/or mesodermal cells. Ligands recognizing the Flt4 receptor tyrosine kinase were purified from a PC-3 prostatic adenocarcinoma cell line (ATCC CRL1435). When applied to a population of cells expressing the Flt4 receptor, ligands of the invention stimulate autophosphorylation, resulting in receptor activation. The invention also provides inhibitors of the Flt4 receptor, including antibodies directed against the receptor. A ligand according to the invention may be coexpressed as a larger precursor which is cleaved to produce the ligand. A coexpressed region in some cases results from alternative splicing of

RNA of the ligand gene. Such a co-expressed region may be a function of the particular expression system used to obtain the ligand. The skilled artisan understands that in recombinant production of proteins, additional sequence may be expressed along with a functional peptide depending upon the particular recombinant construct used to express the protein, and subsequently removed to obtain the desired ligand. In some cases the recombinant ligand can be made lacking certain residues of the endogenous/natural ligand. Moreover, it is well-known in that conservative replacements may be made in a protein which do not alter the function of the protein. Accordingly, it is anticipated that such alterations are within the scope of the claims. It is intended that the precursor sequence shown in SEQ ID NO: 33 is capable of stimulating the Flt4 ligand without any further processing in a manner similar to that in which VEGF stimulates its receptor in its unprocessed form.

The following Examples illustrate preferred embodiments of the invention, wherein the isolation, characterization, and function of Flt4 ligands according to the invention is shown.

EXAMPLE 1

Production of pLTRFlt4l expression vector

Construction of the LTR-Flt4l vector is schematically shown in Figure 2. The full-length Flt4s cDNA (Genbank Accession No. X68203) was assembled by first subcloning the S2.5 fragment, reported in Pajusola *et al.*, *Cancer Res.* 52:5738-5743 (1992), incorporated by reference herein, containing base pairs 56-2534 of the Flt4s into the *EcoRI* site of the pSP73 vector (Promega, Madison, WI).

Since cDNA libraries used for screening of Flt4 cDNAs did not contain its most 5' protein-coding sequences, inverse PCR was used for the amplification of the 5' end of Flt4 corresponding to the first 12 amino acid residues (MQRGAALCLRLW). PolyA⁺ RNA was isolated from the

HEL cells and double-stranded cDNA copy was synthesized using the Amersham cDNA Synthesis System Plus kit and a gene specific primer: 5'-TGTCCTCGCTGTCCTTGTCT-3' (SEQ ID NO: 1), which was located 195 bp downstream of the 5' end of clone S2.5. Double stranded cDNA was treated with T4 DNA polymerase to blunt the ends and cDNA was purified with Centricon 100 (Amicon Inc., Beverly, MA). Circularization was made in a total volume of 150 ul. The reaction mixture contained ligation buffer, 5% PEG-8000, 1 mM DTT and 8U of T4 DNA ligase (New England Biolabs). Ligation was carried out at 16°C for 16 hours. Fifteen µl of this reaction mix was used in a standard 100 ul PCR reaction containing 100 ng of specific primers including *SacI* and *PstI* restriction sites, present in this segment of the Flt4 cDNA, and 1 unit of Taq DNA polymerase (Perkin Elmer Cetus). Two rounds of PCR were performed using 33 cycles (denaturation at 95°C for 1 minute, annealing at 55°C for 2 minutes and elongation at 72°C for 4 minutes). The PCR mixture was treated sequentially with the *SacI* and *PstI* restriction enzymes and after purification with MagicPCR Preps (Promega) DNA fragments were subcloned into the pGEM3Zf(+) vector for sequencing. The sequence obtained corresponds to the 5' end of the Flt4s cDNA clone deposited in the Genbank Database as Accession No. X68203.

The sequence encoding the first 12 amino acid residues was added to the expression construct by ligating an *SphI* digested PCR fragment amplified using reverse transcription-PCR of polyA⁺ RNA isolated from the HEL cells using the oligonucleotides 5'-ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG GACTCCTGGA-3' (SEQ ID NO: 2)(forward primer, *SphI* site underlined, the translational start codon marked in bold follows an optimized Kozak consensus sequence Kozak, *Nucl. Acids Res.* 15: 8125-8148, 1987) and 5'-ACATGCATGC CCCGCCGGT CATCC-3' (SEQ ID NO: 3) (reverse primer, *SphI* site underlined) to the 5' end of the S2.5

fragment, thus replacing unique *SphI* fragment of the S2.5 plasmid. The resulting vector was digested with *EcoRI* and *ClaI* and ligated to a 138 bp PCR fragment amplified from the 0.6 kb *EcoRI* fragment (base pairs 3789 to 4416 in the Genbank X68203 sequence) which encodes the 3' end of

5 Flt4s shown in Figure 1 of Pajusola *et al.*, *Cancer Res.* 52:5738-5743, 1992, using the oligonucleotides 5'-CGGAATTC³ CATGACCCCA AC-3' (SEQ ID NO: 4) (forward, *EcoRI* site underlined) and 5'-CCATCGAT³GG ATCCTACCTG AAGCCGCTTT CTT-3' (SEQ ID NO: 5) (reverse, *ClaI* site underlined). The coding domain was completed by

10 ligation of the 1.2 kb *EcoRI* fragment (base pairs 2535-3789 of sequence X68203) into the above construct. The complete cDNA was subcloned as a *HindIII-ClaI*(blunted) fragment (this *ClaI* site was also included in the 3' primer used to construct the 3' end of the coding sequence) to the pLTRpoly expression vector reported in Mäkelä *et al.*, *Gene*, 118: 293-294

15 (1992) (Genbank accession number X60280), incorporated by reference herein, using its *HindIII-Acc I*(blunted) restriction sites.

The long form of Flt4 was produced by replacing the 3'-end of the short form as follows: The 3' region of the Flt4l cDNA was PCR-amplified using a gene specific and a pGEM 3Z vector specific (SP6

20 promoter) oligonucleotide 5'-ATTTAGGTGACACTATA-3' (SEQ ID NO: 6) as reverse and forward primers, respectively, and an Flt4l cDNA clone containing a 495 bp *EcoRI* fragment extending downstream of the *EcoRI* site at nucleotide 3789 of the Genbank X68203 sequence (the sequence downstream of this *EcoRI* site is deposited as the Flt4 long form 3'

25 sequence having Genbank accession number S66407). The gene specific oligonucleotide contained a *BamHI* restriction site located right after the end of the coding region. The sequence of that (reverse primer) oligonucleotide was 5'-

CCATCGAT³GGATCCCGATGCTGCTTAGTAGCTGT-3' (SEQ ID NO: 7)(*BamHI* site is underlined). The PCR product was digested with *EcoRI*

30

and *Bam*HI and transferred in frame to LTRFlt4s vector fragment from which the coding sequences downstream of the *Eco*RI site at base pair 2535 (see sequence X68203) had been removed by *Eco*RI-*Bam*HI digestion. Again, the coding domain was completed by ligation of the 1.2 kb *Eco*RI
5 fragment (base pairs 2535-3789 of sequence X68203) back into the resulting construct.

EXAMPLE 2

Production and analysis of Flt4I transfected cells

NIH3T3 cells (60 % confluent) were cotransfected with 5^{μg}
10 of the pLTRFlt4I construct and 0.25^{μg} of the pSV2neo vector (ATCC) containing the neomycin phosphotransferase gene, using the DOTAP liposome-based transfection reagents (Boehringer Mannheim, Mannheim, Germany). One day after the transfection the cells were transferred into selection media containing 0.5 mg/ml geneticin (GIBCO, Grand Island, N.Y.). Colonies of geneticin-resistant cells were isolated and analysed for
15 expression of the Flt4 proteins. Cells were lysed in boiling lysis buffer containing 3.3% SDS (sodium dodecyl sulphate), 125 mM Tris, pH 6.8. Protein concentrations of the samples were measured by the BCA method (Pierce, Rockford, IL). About 50^{μg} protein of each lysate was analysed
20 for the presence of Flt4 by 6% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using antisera against the carboxyl terminus of Flt4 and the ECL method (Amersham).

For production of anti-Flt4 antiserum the Flt4 cDNA fragment encoding the 40 carboxyterminal amino acid residues of the short
25 form: NH₂-PMTPTTYKG SVDNQTDSGM VLASEEFEQI ESRHRQESGFR-COOH (SEQ ID NO: 8) was cloned as a 657 bp *Eco*RI-fragment into the pGEX-1IT bacterial expression vector (Pharmacia) in frame with the glutathione-S-transferase coding region. The resulting GST-Flt4S fusion protein was produced in *E. coli* and purified by affinity

chromatography using a glutathione-Sepharose 4B column. The purified protein was lyophilized, dissolved in phosphate buffered saline (PBS), mixed with Freund's adjuvant and used for immunization of rabbits at biweekly intervals using methods standard in the art (Harlow and Lane, 5 Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988). Antisera were used after the fourth booster immunization for immunoprecipitation of Flt4 from the transfected cells and clones expressing Flt4 were used for ligand stimulation analysis.

EXAMPLE 3

10 Construction of a Flt4 EC baculovirus vector and expression and purification of its product

The construction of an Flt4 extracellular domain (EC) baculovirus vector is schematically shown in Figure 3. The Flt4-encoding cDNA has been prepared in both a long form and a short form, each being 15 incorporated in a vector under control of the Moloney murine leukemia virus LTR promoter. The nucleotide sequence of the short form of the Flt4 receptor is available on the Genbank database as Accession No. X68203 and the specific 3' segment of the long form cDNA is available as Accession No. S66407.

20 The ends of a cDNA segment encoding Flt4 extracellular domain (EC) were modified as follows: The 3' end of Flt4 cDNA sequence (Genbank Accession Number ^XX68203) which encodes the extracellular domain was amplified using primer 1116 5'-CTGGAGTCGACTTGGCGGACT-3' (SEQ ID NO: 9, *SaII* site underlined) and primer 1315 5'-CGCGGATCCCTAGTGATGGTGATGGTGATGTCTACCTTCGATCATG 25 CTGCCCTTAT CCTC-3' (SEQ ID NO: 10, *BamHI* site underlined). The sequence complementary to that of primer 1315 continues after the Flt4 reading frame and encodes 6 histidine residues for binding to a Ni-NTA

column (Qiagen, Hilden, Germany) followed by a stop codon, and an added Bam HI site. The amplified fragment was digested with *Sall* and *BamHI* and used to replace a unique *Sall-BamHI* fragment in the LTRFlt4 vector shown in Figure 3. The *Sall-BamHI* fragment that was replaced encodes the Flt4 transmembrane and cytoplasmic domains.

The 5' end without the Flt4 signal sequence encoding region was amplified by PCR using the primer 1335 5'-

CCCAAGCTTGGATCCAAGTGGCTACTCCATGACC-3' (SEQ ID NO: 11) (the primer contains added *HindIII* (AAGCTT) and *BamHI* (GGATCC)

restriction sites, which are underlined) and primer 1332 5'-

GTTGCCTGTGATGTGCACCA-3' (SEQ ID NO: 12). The amplified fragment was digested with *HindIII* and *SphI* (the *HindIII* site (AAGCTT) is underlined in primer 1335 and the *SphI* site is within the amplified region of the Flt4l cDNA). The resulting *HindIII-SphI* fragment was used

to replace a *HindIII-SphI* fragment in the modified LTRFlt4l vector described immediately above (the *HindIII* site is in the 5' junction of the Flt4 insert with the pLTRpoly portion of the vector, the *SphI* site is in Flt4 cDNA). The resulting Flt4EC insert was then ligated as a *BamHI* fragment into the *BamHI* site in the pVTBac plasmid as disclosed in Tessier *et al.*,

Gene 98: 177-183 (1991), incorporated by reference herein. The orientation was confirmed to be correct by partial sequencing so that the open reading frame of the signal sequence-encoding portion of the vector continued in frame with the Flt4 sequence. That construct was transfected together with the baculovirus genomic DNA into SF-9 cells by lipofection.

Recombinant virus was purified, amplified and used for infection of High-Five cells (Invitrogen, San Diego, CA) using methods standard in the art.

The Flt4 extracellular domain was purified from the culture medium of the infected High-Five cells using Ni-NTA affinity chromatography according to manufacturer's instructions (Qiagen) for binding and elution of the 6xHis tag encoded in the COOH-terminus of the recombinant Flt4 extracellular

domain.

EXAMPLE 4

Isolation of Flt4 Ligand from Conditioned Media

An Flt4 ligand according to the invention was isolated from
5 conditioned media from PC-3 prostatic adenocarcinoma cell line CRL1435
from the American Type Culture Collection and cultured as instructed by
the supplier in Ham's F-12 Nutrient mixture (GIBCO) containing 7% fetal
calf serum. In order to prepare the conditioned media, confluent PC-3 cells
10 were cultured for 7 days in Ham's F-12 Nutrient mixture (GIBCO) in the
absence of fetal bovine serum. Medium was then cleared by centrifugation
at 10,000 g for 20 minutes. The medium was then screened to determine
its ability to induce tyrosine phosphorylation of Flt4 by exposure to
NIH3T3 cells which had been transfected with Flt4-encoding cDNA using
the pLTRFlt4l vector. For receptor stimulation experiments, subconfluent
15 NIH3T3 cells were starved overnight in serum-free DMEM medium
(GIBCO) containing 0.2% BSA. The cells were stimulated with the
conditioned media for 5 minutes, washed twice with cold PBS containing
100 uM vanadate and lysed in RIPA buffer (10 mM Tris pH 7.5, 50 mM
NaCl, 0.5% sodium deoxycholate, 0.5% Nonidet P40 (BDH, Poole,
20 England), 0.1% SDS, 0.1 U/ml Aprotinin (Boehringer Mannheim), 100
uM vanadate) for receptor immunoprecipitation analysis. The lysates were
centrifuged for 20 minutes at 15,000 x g. The supernatants were incubated
for 2 hours on ice with 3 ul of the antiserum against the Flt4 C-terminus
described in Example 2 and also in Pajusola, *et al. Oncogene* 8: 2931-
25 2937, (1993), incorporated by reference herein.

After a 2 hour incubation in the presence of anti-Flt4
antiserum, protein A-Sepharose (Pharmacia) was added and incubation was
continued for 45 minutes with rotation. The immunoprecipitates were
washed three times with the immunoprecipitation buffer and twice with 10

mM Tris, pH7.5 before analysis in SDS-PAGE. Polypeptides were transferred to nitrocellulose and analyzed by Western blotting using Flt4- or phosphotyrosine-specific antisera and the ECL method (Amersham International, Buckinghamshire, England). Anti-phosphotyrosine
5 monoclonal antibodies (anti-PTyr; PY20) were purchased from Transduction Laboratories (Lexington, Kentucky). In some cases, the filters were restained with a second antibody after stripping. The stripping of the filters was done for 30 minutes at 50°C in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7 with occasional agitation.

10 As shown in Figure 4, the PC-3 cell conditioned medium stimulated tyrosine phosphorylation of a 125 kD polypeptide when Flt4 expressing NIH3T3 cells were treated with the indicated preparations of media, lysed, and the lysates were immunoprecipitated with anti-Flt4 antiserum followed by SDS-PAGE, Western blotting, and staining using
15 anti-PTyr antibodies. The resulting band was weakly phosphorylated upon stimulation with unconcentrated PC-3 conditioned medium (lane 2). The 125 kD band comigrated with the tyrosine phosphorylated, processed form of the mature Flt4 from pervanadate-treated cells (compare lanes 2 and 7 of Fig. 4, see also Figure 5A). Comigration was confirmed upon restaining
20 with anti-Flt4 antibodies as is also shown in Figure 5A (panel on the right). In order to show that the 125 kD polypeptide is not a non-specific component of the conditioned medium reactive with anti-phosphotyrosine antibodies, 15 ul of conditioned medium was separated by SDS-PAGE, blotted on nitrocellulose and the blot was stained with anti-PTyr antibodies.
25 No signal was obtained (Fig. 5B). Also, unconditioned medium failed to stimulate Flt4 phosphorylation, as shown in Figure 4, lane 1.

As shown in Figure 4, lane 3, stimulating activity was considerably increased when the PC-3 conditioned medium was concentrated four-fold using a Centricon-10 concentrator (Amicon). Figure
30 4, lane 4, shows that pretreatment of the concentrated PC-3 conditioned

medium with 50 ul of the Flt4 extracellular domain coupled to CNBr-activated sepharose CL-4B (Pharmacia; about 1mg of Flt4 EC domain/ml sepharose resin) completely abolished Flt4 tyrosine phosphorylation. Similar pretreatment of the conditioned medium with unsubstituted
5 sepharose CL-4B did not affect stimulatory activity, as shown in Figure 4, lane 5. Also, the flow through obtained after concentration, which contained proteins of less than 10,000 molecular weight, did not stimulate Flt4 phosphorylation, as shown in Figure 4, lane 6.

The foregoing data show that PC-3 cells produce a ligand
10 which binds to the extracellular domain of Flt4 and activates this receptor.

EXAMPLE 5

Purification of the Flt4 Ligand

The ligand expressed by PC-3 cells as characterized in Example 3 was purified and isolated using a recombinant^{ly} produced Flt4
15 extracellular domain in affinity chromatography.

Two harvests of serum-free conditioned medium, comprising a total of 8 L were collected from 500 confluent 15 cm diameter culture dishes containing confluent layers of PC-3 cells. The conditioned medium was clarified by centrifugation at 10,000 x g and concentrated 80-fold using
20 an Ultrasette Tangential Flow Device (Filtron, Northborough, MA) with a 10 kD cutoff Omega Ultrafiltration membrane according to the manufacturer's instruction. Recombinant Flt4 extracellular domain was expressed in a recombinant baculovirus cell system and purified by affinity chromatography on Ni-agarose (Ni-NTA affinity column obtained from
25 Qiagen). The purified extracellular domain was coupled to CNBr-activated Sepharose CL-4B at a concentration of 5 mg/ml and used as an affinity matrix for ligand affinity chromatography.

Concentrated conditioned medium was incubated with 2 ml of the recombinant Flt4 extracellular domain-Sepharose affinity matrix in a

rolling tube at room temperature for 3 hours. All subsequent purification steps were at +4 °C. The affinity matrix was then transferred to a column (Pharmacia) with an inner diameter of 15 mm and washed successively with 100 ml of PBS and 50 ml of 10 mM Na-phosphate buffer (pH 6.8).

- 5 Bound material was eluted step-wise with 100 mM glycine-HCl, successive 6 ml elutions having pHs of 4.0, 2.4, and 1.9. Several 2 ml fractions of the eluate were collected in tubes containing 0.5 ml 1 M Na-phosphate (pH 8.0). Fractions were mixed immediately and dialysed in 1 mM Tris-HCl (pH 7.5). Aliquots of 75 ul each were analyzed for their ability to
- 10 stimulate tyrosine phosphorylation of Flt4. The ultrafiltrate, 100 ul aliquots of the concentrated conditioned medium before and after ligand affinity chromatography, as well as 15-fold concentrated fractions of material released from the Flt4 extracellular domain-Sepharose matrix during the washings were also analyzed for their ability to stimulate Flt4
- 15 tyrosine phosphorylation.

- As shown in Figure 6, lane 3, the concentrated conditioned medium induced prominent tyrosine phosphorylation of Flt4 in transfected NIH3T3 cells overexpressing Flt4. This activity was not observed in conditioned medium taken after medium was exposed to the Flt4
- 20 Sepharose affinity matrix described above (lane 4). The specifically-bound Flt4-stimulating material was retained on the affinity matrix upon washes in PBS, 10 mM Na-phosphate buffer (pH 6.8), and at pH 4.0 (lanes 5-7, respectively), and it was eluted in the first two 2 ml aliquots at pH 2.4 (lanes 8 and 9). A further decrease of the pH of the elution buffer did not
- 25 cause release of additional Flt4-stimulating material (lane 11).

- Small aliquots of the chromatographic fractions were concentrated in a SpeedVac concentrator (Savant, Farmingdale, N.Y.) and subjected to SDS-PAGE under reducing conditions with subsequent silver staining of the gcl. As shown in Figure 7, the major polypeptide, having a
- 30 molecular weight of approximately 23 kD (reducing conditions), was

detected in the fractions containing Flt4 stimulating activity (corresponding to lanes 8 and 9). That polypeptide was not found in the other chromatographic fractions. On the other hand, all other components detected in the two active fractions were also distributed in the starting material and in small amounts in the other washing and elution steps after their concentration. Similar results were obtained in three independent affinity purifications, indicating that the 23 kD polypeptide specifically binds to Flt4 and induces its tyrosine phosphorylation.

Fractions containing the 23 kD polypeptide were combined, dried in a SpeedVac concentrator and subjected to SDS-PAGE in a 12.5 % gel. The proteins from the gel were then electroblotted to Immobilon-P (PVDF) transfer membrane (Millipore, Malborough, MA) and visualized by staining of the blot with Coomassie blue R-250. The region containing only the stained 23 kD band was cut from the blot and was subjected to N-terminal amino acid sequence analysis in a Prosite Protein Sequencing System (Applied Biosystems, Foster City, CA). The data were analyzed using a 610A Data Analysis System (Applied Biosystems). Analysis revealed a single N-terminal sequence of NH₂-XEETIKFAAAHYNTEILK-COOH (SEQ ID NO: 13).

EXAMPLE 6

Construction of PC-3 cell cDNA library in a eukaryotic expression vector.

Poly-A⁺ RNA was isolated from five 15 cm diameter confluent dishes of PC-3 cells by a single step method using oligo(dT) (Type III, Collaborative Research) cellulose affinity chromatography (Sambrook et al., Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989). The yield was 70 µg. Six µg of the poly-A⁺ RNA was used to prepare an oligo(dT)-primed cDNA library in the mammalian expression vector pcDNA I and the Librarian kit of Invitrogen

according to the instructions included in the kit. The library was estimated to contain about 10^6 independent recombinants with an average insert size of approximately 1.8 kb.

EXAMPLE 7

5 Amplification of the unique nucleotide sequence encoding the Flt4 ligand

Degenerate oligonucleotides were designed based on the N-terminal amino acid sequence of the isolated Flt4 ligand and were used as primers in a polymerase chain reaction (PCR) to amplify cDNA encoding the Flt4 ligand from a PC-3 cell library.

10 The PCR was carried out using $1 \mu\text{g}$ of DNA from the amplified PC-3 cDNA library and a mixture of sense-strand primers comprising 5'-GCAGARGARACNATHAA-3' (SEQ ID NO: 14) (wherein R is A or G, N is A, G, C or T and H is A, C or T), encoding amino acid residues 2-6 (EETIK, SEQ ID NO: 15) and antisense-strand primers 5'-
15 GCAYTTNARDATYTCNGT-3' (SEQ ID NO: 16) (wherein Y is C or T and D is A, G or T), corresponding to amino acid residues 14-18 (TEILK, SEQ ID NO: 17). Three extra nucleotides (GCA) were added to the 5'-terminus of each primer to increase annealing stability. Two successive
20 PCR runs were carried out using 1U per reaction of DynaZyme, a thermostable DNA polymerase (F-500L, Finnzymes), in a buffer supplied by the manufacturer (10 mM Tris-HCl, pH 8.8 at 25 °C, 1.5 mM MgCl_2 , 50 mM KCl, 0.1% Triton-X100) at an extension temperature of 72 °C. The first PCR run was carried out for 43 cycles. The first three cycles
25 were run at annealing temperature 33 °C for 2 minutes and the remaining cycles were run at 42 °C for 1 minute.

The region of the gel containing a weak band of the expected size (57 bp) was cut out from the gel and eluted. The eluted material was reamplified for 30 cycles using the same primer pairs described above at

42°C for 1 minute. The amplified fragment was cloned into a pCR II vector (Invitrogen) using the TA cloning kit (Invitrogen) and sequenced using the radioactive dideoxynucleotide sequencing method of Sanger. Six clones were analysed and all contained the sequence encoding the expected peptide (amino acids 2-18 of the Flt4 ligand precursor). Nucleotide sequence spanning the region from the third nucleotide of codon 6 to the third nucleotide of codon 13 (the extension region) was identical in all six clones: 5'-ATTCGCTGCAGCACACTACAAC-3' (SEQ ID NO: 18) and thus was considered to represent an amplified product from the unique sequence encoding part of the amino terminus of the Flt4 ligand.

EXAMPLE 8

Amplification of the 5'-end of the cDNA encoding the Flt4 ligand

Based on the unique nucleotide sequence encoding the N-terminus of the isolated Flt4 ligand, two pairs of nested primers were design to amplify in two subsequent PCR-reactions the complete 5'-end of the corresponding cDNAs from 1 µg of DNA from the above-described PC-3 cDNA library. First, amplification was performed with primer 5'-TCNGTGTTGTAGTGTGCTG-3' (SEQ ID NO: 19) which is the antisense-strand primer corresponding to amino acid residues 9-15 (AAHYNTE, SEQ ID NO: 20) and sense-strand primer 5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO: 21) corresponding to the T7 RNA promoter of the pcDNAI vector used for construction of the library. "Touchdown" PCR was used as disclosed in Don, *et al.*, *Nucl. Acids Res.*, 19: 4008 (1991), incorporated by reference herein. The annealing temperature of the two first cycles was 62 °C and subsequently the annealing temperature was decreased in every other cycle by 1 °C until a final temperature of 53 °C was reached, at which temperature 16 additional cycles were carried out. Annealing time was 1 minute and extension at each cycle was conducted at 72 °C for 1

minute. Multiple amplified DNA fragments were obtained in the first reaction. The products of the first amplification (1 ul of a 1:100 dilution in water) were used in the second amplification reaction employing the nested primers 5'-GTTGTAGTGTGCTGCAGCGAATTT-3' (SEQ ID NO: 22), an
5 antisense-strand primer corresponding to amino acid residues 6-13 (KFAAAHYN, SEQ ID NO: 23) of the Flt4 ligand and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 24), a sense-strand primer corresponding to nucleotides 2179-2199 of the pcDNAI vector. The sequences of these sense and antisense primers overlapped with the 3' ends
10 of the corresponding primers used in the first PCR. "Touchdown" PCR was carried out by decreasing the annealing temperature from 72 °C to 66 °C and continuing with 18 additional cycles at 66 °C. The annealing time was 1 minute and extension at each cycle was carried out at 72 °C for 2 minutes. One major product of about 220 bp and three minor products of
15 about 270 bp, 150 bp, and 100 bp were obtained.

The amplified fragment of approximately 220 bp was cut out from the agarose gel, cloned into a pCRII vector using the TA cloning kit (Invitrogen) and sequenced. Three recombinant clones were analysed and they contained the sequence 5'-
20 TCACTATAGGGAGACCCAAGCTTGGTACCGAGCTCGGATCCACTA
GTAACGGCCGCCAGTGTGGTGGAATTCGACGAACTCATGACTGTA
CTCTACCCAGAATATTGGAAAATGTACAAGTGTCAGCTAAGGCAA
GGAGGCTGGCAACATAACAGAGAACAGGCCAACCTCAACTCAAG
GACAGAAGAGACTATAAAAATTCGCTGCAGCACACTACAAC- 3'
25 (SEQ ID NO: 25). The beginning of the sequence represents the pcDNAI vector and the underlined sequence represents the amplified product of the 5'-end of the insert. The ATG codon located upstream of that sequence in the same reading frame is followed by an open reading frame containing the amplified product of the putative signal sequence and the first 13
30 amino acid residues of the secreted Flt4 ligand.

EXAMPLE 9

Amplification of the 3'-end of cDNA encoding the Flt4 ligand

Based upon the amplified 5'-sequence of the clones encoding the Flt4 ligand, two pairs of non-overlapping nested primers were designed to amplify the 3'-portion of the clones. The sense-strand primer 5'-ACAGAGAACAGGCCAACC-3' (SEQ ID NO: 26) and antisense-strand primer 5'-TCTAGCATTTAGGTGACAC-3' (SEQ ID NO: 27) corresponding to nucleotides 2311-2329 of the pcDNAI vector were used in a first "touchdown" PCR. The annealing temperature of the reaction was decreased 1°C every two cycles from 72°C to 52°C, at which temperature 15 additional cycles were carried out. The annealing time was 1 minute and extension at each cycle was carried out at 72°C for 3 minutes. DNA fragments of several sizes were obtained in the first amplification. Those products were diluted 1:200 in water and reamplified in PCR using the second pair of primers: 5'-AAGAGACTATAAAATTCGCTGCAGC-3' (SEQ ID NO: 28) and 5'-CCCTCTAGATGCATGCTCGA-3' (SEQ ID NO: 29) (antisense-strand primer corresponding to nucleotides 2279-2298 of the pcDNAI vector). Two DNA fragments were obtained, having sizes of 1350 bp and 570 bp. Those fragments were cloned into a pCRII vector and the inserts of the clones were sequenced. Both of these fragments were found to contain sequences encoding an amino acid sequence homologous to the VEGF sequence.

EXAMPLE 10

Screening the PC-3 cell cDNA library using the 5' PCR fragment of Flt4 ligand cDNA

A 219 bp 5'-terminal fragment of Flt4 ligand cDNA was amplified by PCR using the 5' PCR fragment described above and primers 5'-GTTCTAGTGTGCTGCAGCGAATTT-3' (antisense-strand primer, SEQ ID NO: 30) and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO:

31) (sense-primer corresponding to nucleotides 2179-2199 of the pcDNAI vector). The amplified product was subjected to digestion with *EcoRI* (Boehringer Mannheim) to remove the portion of the DNA sequence amplified from the pcDNAI vector and the resulting 153 bp fragment encoding the 5' end of the Flt4 ligand was labeled with [³²P]-dCTP using the Klenow fragment of *E. coli* DNA polymerase I (Boehringer Mannheim). That fragment was used as a probe for hybridization screening of the amplified PC-3 cell cDNA library.

Filter replicas of the library were hybridized with the radioactively labeled probe at 42 °C for 20 hours in a solution containing 50% formamide, ^{5x}SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA. Filters were washed twice in ^{4x}SSC, 0.1% SDS for 30 minutes at room temperature, then twice for 30 minutes at 65 °C and exposed overnight.

On the basis of autoradiography, 10 positive recombinant bacterial colonies hybridizing with the probe were chosen from the library. Plasmid DNA was purified from these colonies and analysed by *EcoRI* and *NorI* digestion and agarose gel electrophoresis followed by ethidium bromide staining. The ten plasmid clones were divided into three groups on the basis of the presence of insert sizes of approximately 1.7, 1.9 and 2.1 kb, respectively. Inserts of plasmids from each group were sequenced using the T7 oligonucleotide as a primer and walking primers for subsequent sequencing reactions.

Sequence analysis showed that all clones contain the open reading frame encoding the NH2-terminal sequence of the Flt4 ligand. Furthermore, the 2.1 and 1.9 kb clones also contained sequences encoding the signal sequence. The 5' end of the 1.7 kb clone began within the signal sequence-encoding portion. Dideoxy sequencing was continued using walking primers in the downstream direction. An 1140 nucleotide portion of the sequence of the longest clone is shown in Figure 9. As can be seen

in that figure, after the putative signal sequence the open reading frame terminates in a TAA stop codon 317 amino acid residues further downstream from the signal sequence. When compared with sequences in the GenBank Database, the predicted protein product of this reading frame was found to be homologous with the predicted amino acid sequences of the PDGF/VEGF family of growth factors, as shown in Figure-10.

EXAMPLE 11

Stimulation of Flt4 autophosphorylation by the protein product of the Flt4 ligand vector

The 2.1 kb insert of the Flt4-L clone in pcDNA1 vector containing the open reading frame encoding the sequence shown in Fig. 9 (SEQ ID NO: 32) was cut out from the vector using *HindIII* and *NotI* restriction enzymes, isolated from a preparative agarose gel and ligated to the corresponding sites in the pREP7 expression vector (Invitrogen). The pREP7 vector containing the above cloned insert was transfected into 293-EBNA cells (Invitrogen) using the calcium phosphate transfection method (Sambrook et al., Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989). About 48 hours after transfection the medium of the transfected cells was changed to DMEM medium lacking fetal calf serum and incubated for 36 h. The thus conditioned medium was then collected, centrifuged at 5000xg for 20 minutes, the supernatant was used concentrated 5-fold using Centriprep 10 (Amicon) and to stimulate NIH3T3 cells expressing LTRFlt41, as in Example 4. The cells were lysed, immunoprecipitated using anti-Flt4 antiserum and analysed by Western blotting using anti-phosphotyrosine antibodies.

As can be seen from Fig. 11, lanes 1 and 3, the conditioned medium from two different dishes of the transfected cells stimulated Flt4 autophosphorylation in comparison with the medium from mock-transfected cells, which gave only background levels of phosphorylation of the Flt4

receptor (lane 2). When the concentrated conditioned medium was preabsorbed with 20 μ l of a slur of Flt4EC domain coupled to Sepharose (see example 4), no phosphorylation was obtained (lane 4), showing that the activity responsible for Flt4 autophosphorylation was indeed the Flt4 ligand. Thus, these results demonstrate that the Flt4-L plasmid vector clone having an approximately 2.1 kb insert and containing the open reading frame shown in Fig-9 is expressed into a Flt4 ligand in cells transfected with the Flt4-L expression vector clone, and thus is biologically active. The sequence encoded by that open reading frame is shown in SEQ ID NO: 33. Plasmid ~~FLT4~~^{Flt4-L} has been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 as accession number 97231.

However, the predicted molecular weight of the mature protein product deduced from this reading frame is 35,724 and the Flt4 ligand from PC-3 cell cultures had an approximate molecular weight of 23 kD under reducing conditions. It is thus possible that the Flt4 mRNA may be first translated into a precursor, from which the mature ligand is derived by proteolytic cleavage. The difference in the observed molecular weight of the isolated Flt4 ligand and the deduced molecular weight of the disclosed open reading frame of the Flt4 ligand sequence may then derive from sequences in the carboxyl terminal region of the latter. Also, the Flt4 ligand may be glycosylated at two putative N-linked glycosylation sites conforming to the consensus which can be identified in the deduced Flt4 ligand amino acid sequence (N-residues underlined marked in Fig- 10).

The carboxyl terminal amino acid sequences, which increase the predicted molecular weight of the Flt4 ligand subunit in comparison with other ligands of this family show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring protein 3 (BRP3) sequence (Dignam and Case, Gene 88, 133-140, 1990). Such a sequence may encode an independently folded domain present in a Flt4 ligand precursor and it

may be involved, for example, in the regulation of secretion, solubility, stability, cell surface localization or activity of the Flt4 ligand.

Interestingly, at least one cysteine motif of the BRP3 type is also found in the VEGF carboxy terminal amino acid sequences.

5 Thus, the Flt4 mRNA may be first translated into a precursor from the mRNA corresponding to the Flt4-L clone, from which the mature ligand is derived by proteolytic cleavage. To define the mature Flt4 ligand product one first expresses the cDNA clone, which is deposited in the pcDNA1 expression vector, in cells, such as COS cells and use
10 antibodies generated against Flt4-L-encoded peptides, such as amino terminal 23 amino acid peptide or bacterial Flt4 fusion proteins, such as a GST-fusion protein, to raise antibodies against the VEGF-homologous domain of Flt4 ligand. One then follows the biosynthesis and processing of the Flt4 ligand in the transfected cells by pulse-chase analysis using
15 radioactive cysteine for labelling of the cells, immunoprecipitation and gel electrophoresis. Using antibodies against the two domains of the product of the Flt4-L clone material for radioactive or nonradioactive aminoterminal sequence analysis is isolated. The determination of the NH2-terminal
20 sequence of the carboxyl terminal fragment allows for identification of the proteolytic processing site. This is confirmed by site-directed mutagenesis of the amino acid residues adjacent to the cleavage site, which would prevent the cleavage.

On the other hand, the Flt4 ligand is characterized by progressive 3' deletions in the 3' coding sequences of the Flt4 ligand
25 precursor clone, resulting in ^{in body}COOH-terminal truncations of its protein product. The activities of such truncated forms are assayed by, for example, studying Flt4 autophosphorylation induced by the truncated proteins when applied to cultures of cells, such ^{as} NIH3T3 cells
30 expressing LTRFlt4. By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin et al.,

8/12/97

Growth Factors 8, 245-252, 1993) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues.

On the other hand, the difference between the molecular
5 weights of the purified ligand and the open reading frame of the Flt4 precursor clone may be due to the fact that the soluble ligand was produced from an alternatively spliced mRNA which would also be present in the PC-3 cells, from which the isolated ligand was derived. To isolate such alternative cDNA clones one uses cDNA fragments of the deposited clone
10 and PCR primers made according to the sequence provided as well as techniques standard in the art to isolate or amplify alternative cDNAs from the PC-3 cell cDNA library. One may also amplify using reverse transcription (RT)-PCR directly from the PC-3 mRNA using the primers provided in the sequence of the Flt4-L clone. Alternative cDNAs can be
15 sequenced from the resulting cDNA clones. One can also isolate genomic clones corresponding to the Flt4-L transcript from a human genomic DNA library using methods standard in the art and to sequence such clones or their subcloned fragments to reveal the corresponding exons. Alternative exons can then be identified by a number of methods standard in the art,
20 such as heteroduplex analysis of cDNA and genomic DNA and they can subsequently be characterized.

EXAMPLE 12

Expression of the Flt4-L gene

Expression of transcripts corresponding to the Flt4 ligand
25 was analysed by hybridization of Northern blots containing isolated polyA⁺ RNA from HT-1080 and PC-3 human tumor cell lines. The probe was the radioactively labelled insert of the 2.1 kb cDNA clone (specific activity 10^8 - 10^9 cpm/mg of DNA). The blot was hybridized overnight at 42°C, using 50% formamide, 5 x SSPE buffer, 2% SDS, 10 x Denhardt's

5 solution, 100 mg/ml salmon sperm DNA and 1×10^6 cpm of the labelled probe/ml. The blot was washed at room temperature for 2 x 30 minutes in 2 x SSC containing 0.05% SDS and then for 2 x 20 min at ^{52°C} in 0.1 x SSC containing 0.1% SDS. The blot was then exposed at ^{-70°C} ~~-70°C~~ for three days using intensifying screens and Kodak XAR film. Both cell lines expressed an Flt4 ligand mRNA of about 2.3 kb, as well as VEGF and VEGF-B mRNA:s (Fig. 12).

- 30 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Alitalo, Kari
Joukov, Vladomir
- (ii) TITLE OF INVENTION: Receptor Ligand
- (iii) NUMBER OF SEQUENCES: 35
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
 - (B) STREET: 6300 Sears Tower, 233 South Wacker Drive
 - (C) CITY: Chicago
 - (D) STATE: Illinois
 - (E) COUNTRY: United States of America
 - (F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/510,133
 - (B) FILING DATE: 08/01/95
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Gass, David A.
 - (B) REGISTRATION NUMBER: 38,153
 - (C) REFERENCE/DOCKET NUMBER: 28113/32863
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 312/474-6300
 - (B) TELEFAX: 312/474-0448
 - (C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTCCTCGCT GTCCTGTCT

20

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 70 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG
GACTCCTGGA

60

70

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACATGCATGC CCCGCCGGTC ATCC

24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGAATTCCC CATGACCCCA AC

22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT

33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATTTAGGTGA CACTATA

17

- 32 -

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT

34

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro	Met	Thr	Pro	Thr	Thr	Tyr	Lys	Gly	Ser	Val	Asp	Asn	Gln	Thr	Asp
1				5					10					15	
Ser	Gly	Met	Val	Leu	Ala	Ser	Glu	Glu	Phe	Glu	Gln	Ile	Glu	Ser	Arg
			20					25					30		
His	Arg	Gln	Glu	Ser	Gly	Phe	Arg								
			35				40								

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGAGTCGA CTTGGCGGAC T

21

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC

60

RECEIVED

RECEIVED 1984-02-20

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC

34

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTTGCCTGTG ATGTGCACCA

20

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
 1 5 10 15
 Leu Lys

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCACGACGACA CATHAA

17

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:

- 34 -

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Glu Thr Ile Lys
1 5

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAYTTNARD ATYTCNGT

18

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Thr Glu Ile Leu Lys
1 5

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTCGCTGCA GCACACTACA AC

22

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- 35 -

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCAGTGTGT AGTGTGCTG

19

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Ala His Tyr Asn Thr Glu
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TAATACGACT CACTATAGGG

20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Phe Ala Ala Ala His Tyr Asn
1 5

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 219 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCACTATAGG GAGACCCAAG CTGGGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT
GTGGTGGAAT TCGACGAACT CATGACTGTA CTCTACCCAG AATATTGGAA AATGTACAAG
TGTCAGCTAA GGCAAGGAGG CTGGCAACAT AACAGAGAAC AGGCCAACCT CAACTCAAGG
ACAGAAGAGA CTATAAAATT CGCTGCAGCA CACTACAAC

60

120

180

219

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACAGAGAACA GGCCAACC

18

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCTAGCATTT AGGTGACAC

19

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
AAGAGACTAT AAAATTCGCT GCAGC

25

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
CCCTCTAGAT GCATGCTCGA

20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
GTTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1140 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 37..1089

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAACTC	ATG ACT GTA CTC TAC CCA	54
	Met Thr Val Leu Tyr Pro	
	1	5
GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA		102
Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln		
	10	15
CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA		150
His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Thr Ile		
	25	30
AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT		198
Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp		
	40	45
AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT		246
Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp		
	55	60
GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA		294
Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro		
	75	80
TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG		342
Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu		
	90	95
CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA		390
Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu		
	105	110
ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT		438
Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe		
	120	125
GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA		486
Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg		
	135	140
CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG		534
Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln		
	155	160
TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT		582
Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn		
	170	175
CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT		630
His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp		
	185	190
GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC		678
Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn		
	200	205

AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu 215 220 225 230	726
CGG CCT GCC AGG TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys 235 240 245	774
CAG TGT GTC TGT AAA AAC AAA CTC TTC CCC AGC CAA TGT GGG GCC AAC Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn 250 255 260	822
CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys 265 270 275	870
CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr 280 285 290	918
GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln 295 300 305 310	966
ACA TGC AGC TGT TAC AGA CGG CCA TGT ACG AAC CGC CAG AAG GCT TGT Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys 315 320 325	1014
GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser 330 335 340	1062
TAT TGG AAA AGA CCA CAA ATG AGC TAAGATTGTA CTGTTTTCCTA GTTCATCGAT Tyr Trp Lys Arg Pro Gln Met Ser 345 350	1116
TTTCTATTAT CGAAACTGT GTTG	1140

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 350 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu 1 5 10 15
Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser 20 25 30
Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu 35 40 45
Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro 50 55 60
Arg Gln Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn 65 70 75 80

- 40 -

Thr	Phe	Phe	Lys	Pro	Pro	Cys	Val	Ser	Val	Tyr	Arg	Cys	Gly	Gly	Cys	85	90	95
Cys	Asn	Ser	Glu	Gly	Leu	Gln	Cys	Met	Asn	Thr	Ser	Thr	Ser	Tyr	Leu	100	105	110
Ser	Lys	Thr	Leu	Phe	Glu	Ile	Thr	Val	Pro	Leu	Ser	Gln	Gly	Pro	Lys	115	120	125
Pro	Val	Thr	Ile	Ser	Phe	Ala	Asn	His	Thr	Ser	Cys	Arg	Cys	Met	Ser	130	135	140
Lys	Leu	Asp	Val	Tyr	Arg	Gln	Val	His	Ser	Ile	Ile	Arg	Arg	Ser	Leu	145	150	155
Pro	Ala	Thr	Leu	Pro	Gln	Cys	Gln	Ala	Ala	Asn	Lys	Thr	Cys	Pro	Thr	165	170	175
Asn	Tyr	Met	Trp	Asn	Asn	His	Ile	Cys	Arg	Cys	Leu	Ala	Gln	Glu	Asp	180	185	190
Phe	Met	Phe	Ser	Ser	Asp	Ala	Gly	Asp	Asp	Ser	Thr	Asp	Gly	Phe	His	195	200	205
Asp	Ile	Cys	Gly	Pro	Asn	Lys	Glu	Leu	Asp	Glu	Glu	Thr	Cys	Gln	Cys	210	215	220
Val	Cys	Arg	Ala	Gly	Leu	Arg	Pro	Ala	Ser	Cys	Gly	Pro	His	Lys	Glu	225	230	235
Leu	Asp	Arg	Asn	Ser	Cys	Gln	Cys	Val	Cys	Lys	Asn	Lys	Leu	Phe	Pro	245	250	255
Ser	Gln	Cys	Gly	Ala	Asn	Arg	Glu	Phe	Asp	Glu	Asn	Thr	Cys	Gln	Cys	260	265	270
Val	Cys	Lys	Arg	Thr	Cys	Pro	Arg	Asn	Gln	Pro	Leu	Asn	Pro	Gly	Lys	275	280	285
Cys	Ala	Cys	Glu	Cys	Thr	Glu	Ser	Pro	Gln	Lys	Cys	Leu	Leu	Lys	Gly	290	295	300
Lys	Lys	Phe	His	His	Gln	Thr	Cys	Ser	Cys	Tyr	Arg	Arg	Pro	Cys	Thr	305	310	315
Asn	Arg	Gln	Lys	Ala	Cys	Glu	Pro	Gly	Phe	Ser	Tyr	Ser	Glu	Glu	Val	325	330	335
Cys	Arg	Cys	Val	Pro	Ser	Tyr	Trp	Lys	Arg	Pro	Gln	Met	Ser			340	345	350

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1997 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CCCCCCCCGC CTCTCAAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCCTCGCC	60
CTCCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCGGTTT CCTGTGAGGC	120
TTTTACCTGA CACCCGCCGC CTTTCCCCGG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG	180
GGAACGCGGA GCGCCGGACC CGCTCCCGCC GCCTCCGGCT CGCCAGGGG GGCTCGCCGG	240
GAGGAGCCCG GGGGAGAGGG ACCAGGAGGG GCGCGCGGCC TCGCAGGGGC GCGCGCGCCC	300
CCACCCCTGC CCGCGCCAGC GGACCGGTCC CCCACCCCGC GTCCTTCCAC C ATG CAC	357
	Met His
	1
TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG	405
Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Ala Leu	
	5 10 15
CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC GCC TTC GAG TCC	453
Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser	
	20 25 30
GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT	501
Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala	
	35 40 45 50
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA	549
Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val	
	55 60 65
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG	597
Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys	
	70 75 80
TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC	645
Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn	
	85 90 95
CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT	693
Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr	
	100 105 110
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA	741
Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln	
	115 120 125
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC	789
Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val	
	135 140 145
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT	837
Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys	
	150 155 160

GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr 165 170 175	885
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln 180 185 190	933
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg 195 200 205 210	981
TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg 215 220 225	1029
CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Lys Thr 230 235 240	1077
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala 245 250 255	1125
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Ser Thr Asp 260 265 270	1173
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr 275 280 285 290	1221
TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro 295 300 305	1269
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys 310 315 320	1317
CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr 325 330 335	1365
TGC CAC TGT CTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn 340 345 350	1413
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu 355 360 365 370	1461
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG Leu Lys Gly Lys Lys Phe His His Glu Thr Cys Ser Cys Tyr Arg Arg 375 380 385	1509
CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser 390 395 400	1557
GAA GAA GTC TTC GTC TTC TTC GTC TTC TTA TAT TGC AAA AGA CTA CAA ATG Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Glu Met 405 410 415	1605

AGC TAAGATTGTA CTGTTTTCCTA GTTCATCGAT TTTCTATTAT GGAAAACCTGT 1658
 Ser
 GTTGCCACAG TAGAACTGTC TGTGAACAGA GAGACCCTTG TGGGTCCATG CTAACAAAGA 1718
 CAAAAGTCTG TCTTTCCTGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGCTCATC 1778
 TGCAAAAGGC CTCCTGTAAA GACTGGTTTT CTGCCAATGA CCAAACAGCC AAGATTTTCC 1838
 TCTTCTCATT TCTTTAAAAG AATGACTATA TAATTTATTT CCACTAAAAA TATTGTTTCT 1898
 GCATTCATTT TTATAGCAAC AACAATTGGT AAAACTCACT GTGATCAATA TTTTATATC 1958
 ATGCAAAATA TGTTTAAAT AAAATGAAAA TTGTATTAT 1997

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 419 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala 1718
 1 5 10
 Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe 1778
 20 25 30
 Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala 1838
 35 40 45
 Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser 1898
 50 55 60
 Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met 1958
 65 70 75 80
 Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln 1997
 85 90 95
 Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala 1718
 100 105 110
 His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys 1778
 115 120 125
 Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Thr 1838
 130 135 140
 Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr 1898
 145 150 155 160
 Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr 1958
 165 170 175
 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu 1997
 180 185 190

- 44 -

Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
195 200 205

Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
210 215 220

Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
225 230 235 240

Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
245 250 255

Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
260 265 270

Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
275 280 285

Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
290 295 300

Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
305 310 315 320

Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
325 330 335

Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
340 345 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
355 360 365

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
370 375 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
385 390 395 400

Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
405 410 415

Gln Met Ser

CLAIMS

Sub 1
1. A purified and isolated peptide which specifically binds to the Flt4 receptor tyrosine kinase.

2. A purified and isolated peptide having the amino acid sequence shown in SEQ ID NO: 33.

3. A nucleic acid encoding the purified and isolated peptide according to claim 2.

4. The nucleic acid according to claim 3 having the sequence shown in SEQ ID NO: 32.

5. A vector comprising the nucleic acid according to claim 4.

Sub 3
6. The vector according to claim 5, wherein said vector is plasmid pFlt4, deposited as ATCC accession No. .

7. A host cell comprising the vector according to claim 6.

Sub 4
8. A fragment of the purified and isolated peptide according to claim 2 which is capable of specifically binding to an Flt4 receptor tyrosine kinase.

9. The fragment according to claim 8 having an apparent molecular weight of 23 kD under reducing conditions.

10. The fragment according to claim 8 comprising approximately the first 180 amino acids shown in SEQ ID NO: 32.

11. An antibody which is specifically reactive with the Flt4 ligand.

~~12. A pharmaceutical composition comprising a peptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.~~

add
C⁵

Add
D₁



08/510133

- 43 -

ABSTRACT

Provided are ligands for the receptor tyrosine kinase, Flt4.
Also provided are cDNAs and vectors encoding the ligand, pharmaceutical compositions and diagnostic reagents.



JOINT INVENTORS

"EXPRESS MAIL" mailing label No.
EG473138672US.

Date of Deposit: August 1, 1995

I hereby certify that this paper (or fee) is
being deposited with the United States Postal
Service "EXPRESS MAIL POST OFFICE TO
ADDRESSEE" service under 37 CFR §1.10 on
the date indicated above and is addressed to:
Assistant Commissioner for Patents,
Washington, D.C. 20231


Thomas C. Meyers

APPLICATION FOR
UNITED STATES LETTERS PATENT

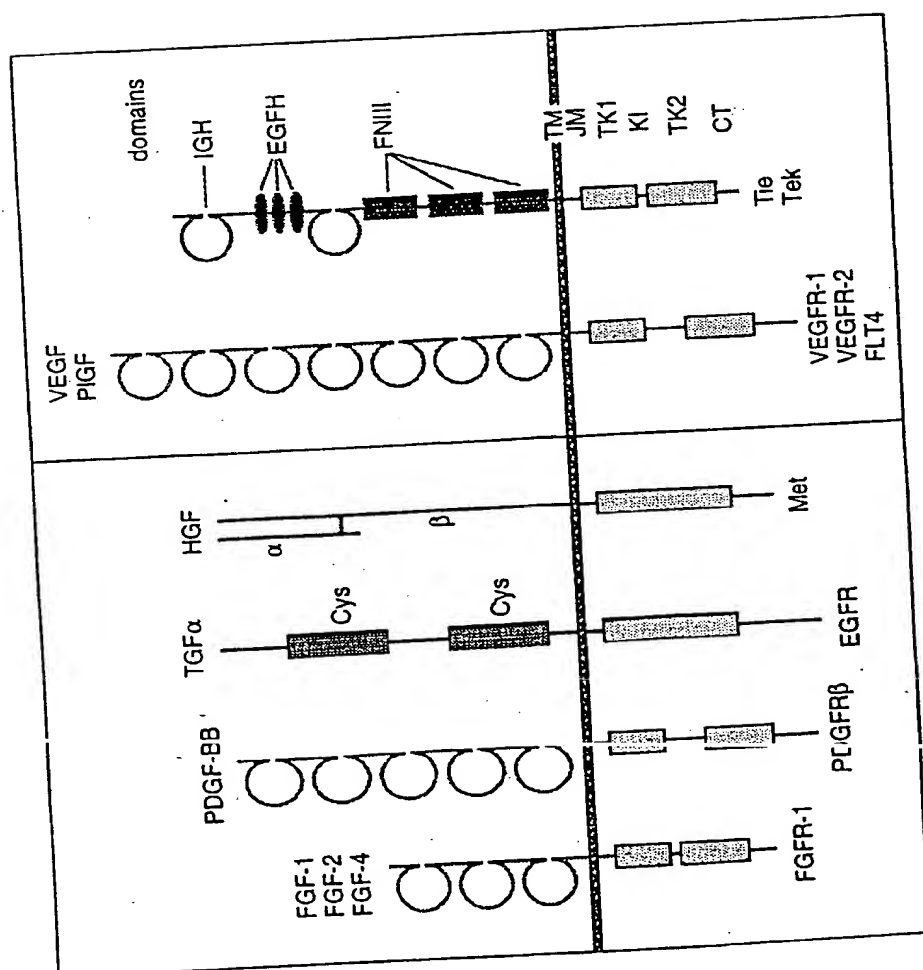
S P E C I F I C A T I O N

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Kari Alitalo a citizen of Finland, residing at
Nyyrikintie 4A, 02100 Espoo, Finland, and Vladimir Joukov a citizen of
Finland, residing at Topeliuksenkatu 32G8, 00290 Helsinki, Finland, have
invented a new and useful "RECEPTOR LIGAND", of which the following is a
specification.

08/510133

FIGURE 1



08/5101

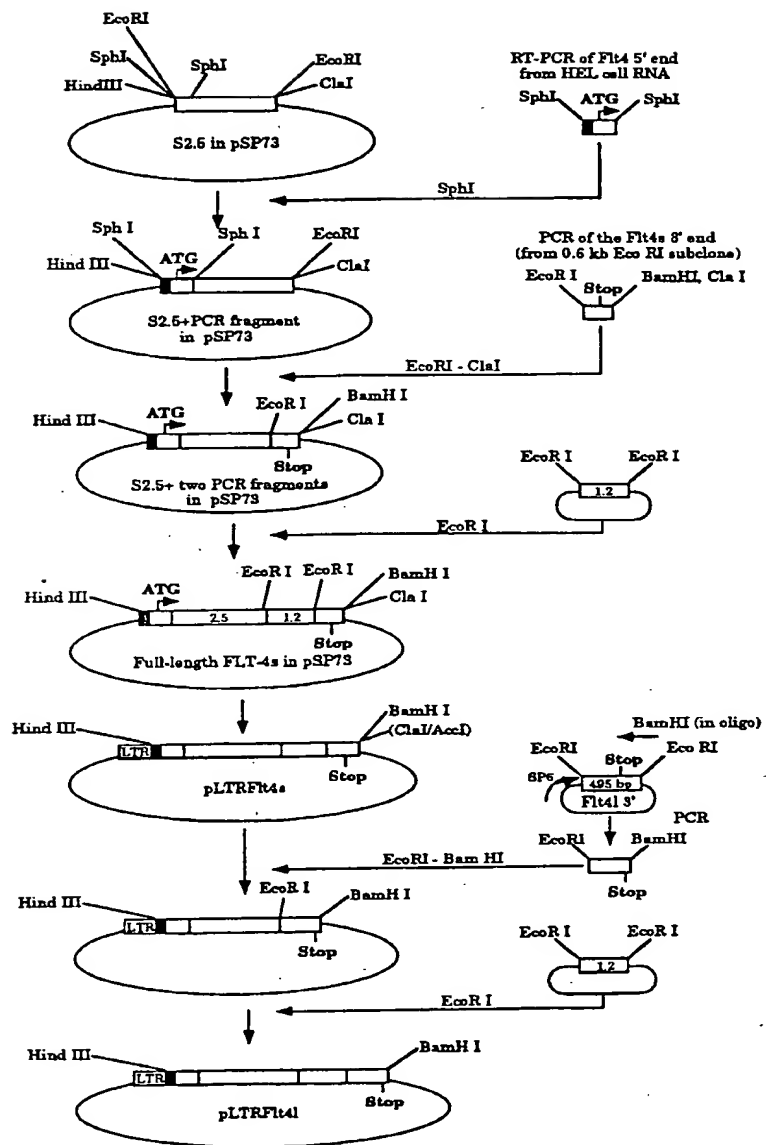
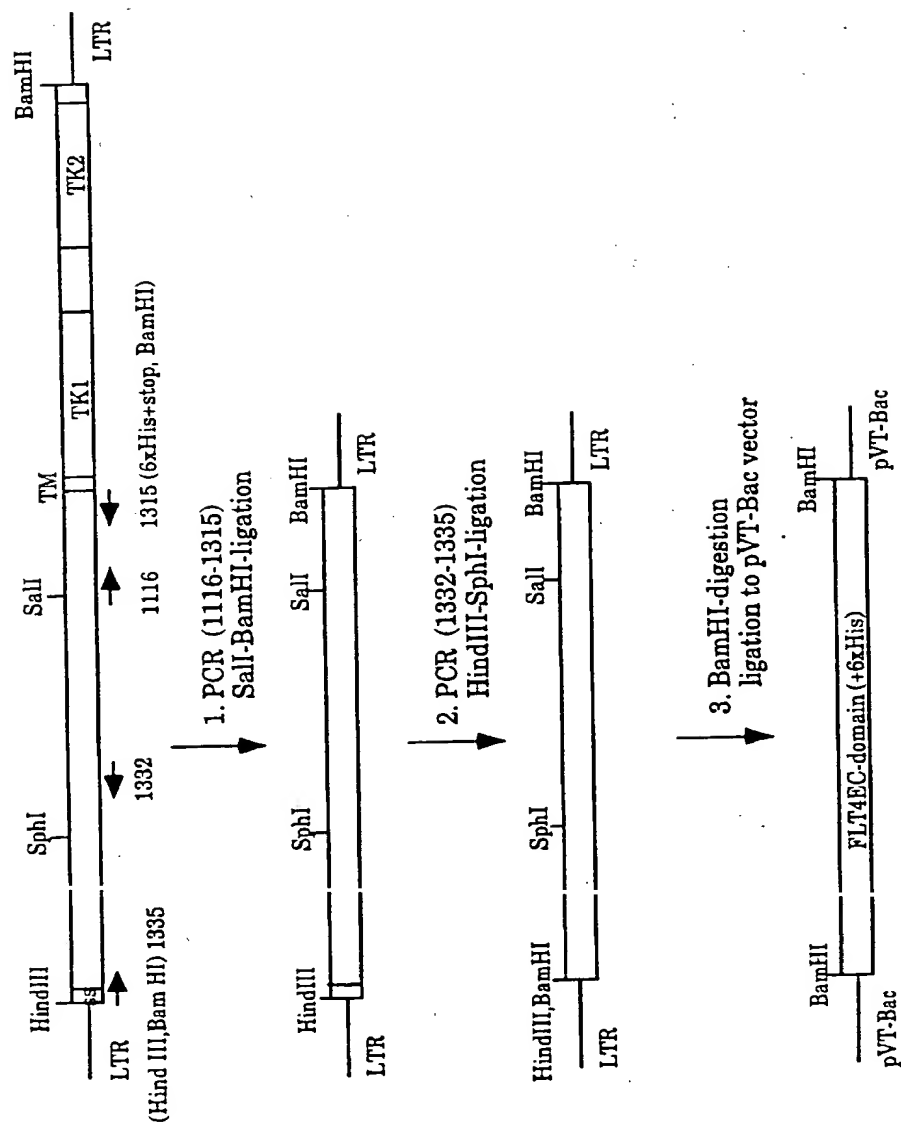


FIGURE 2

Figure 3



08/51013

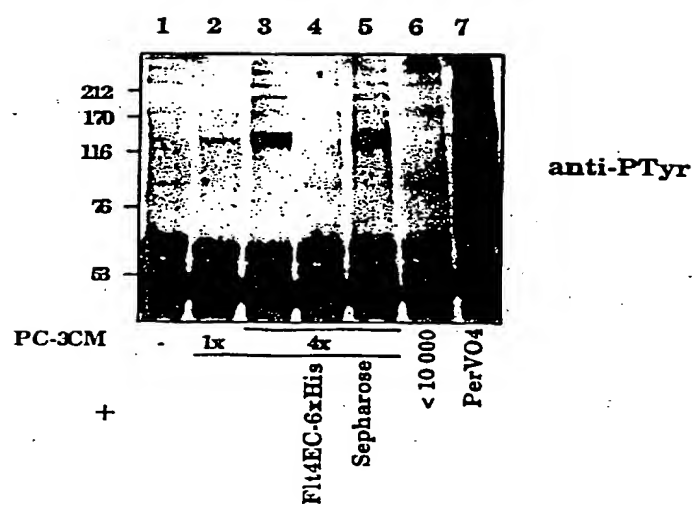


FIGURE 4

08/51013

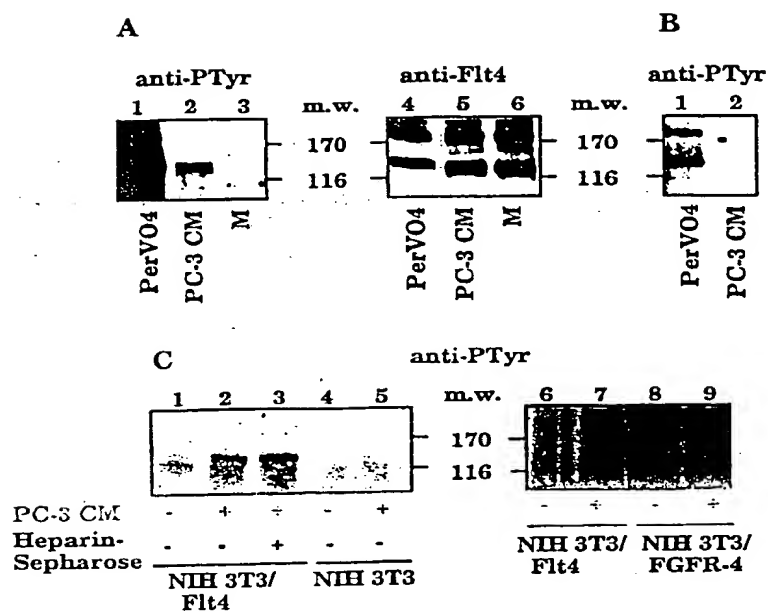
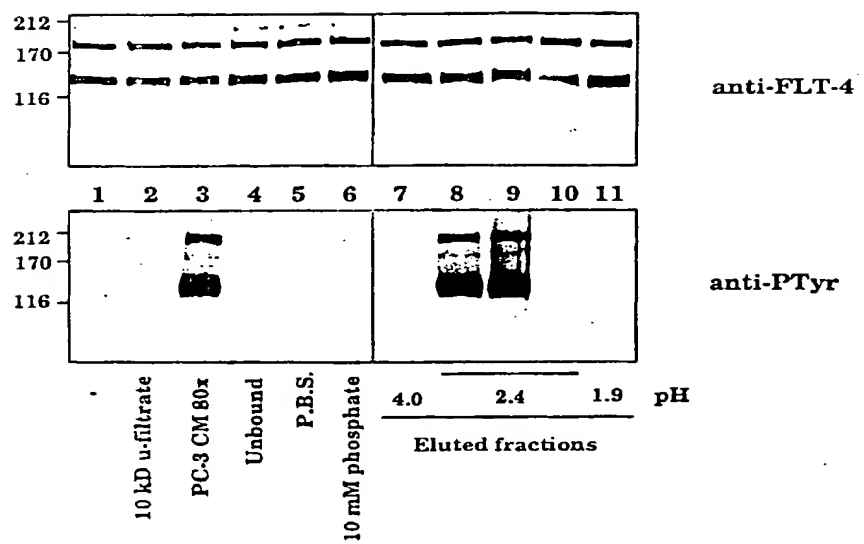


FIGURE 5

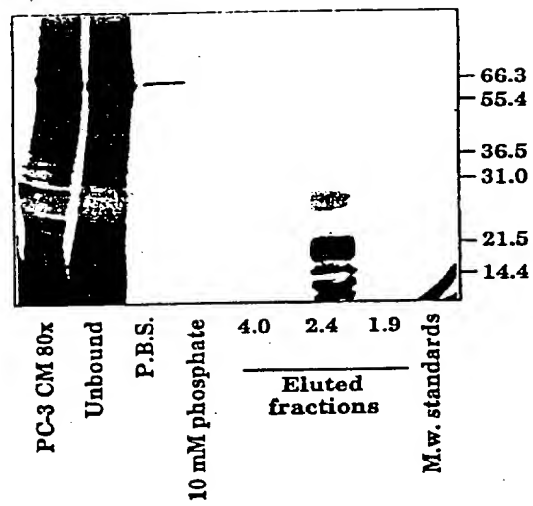
08/51013c

FIGURE 6



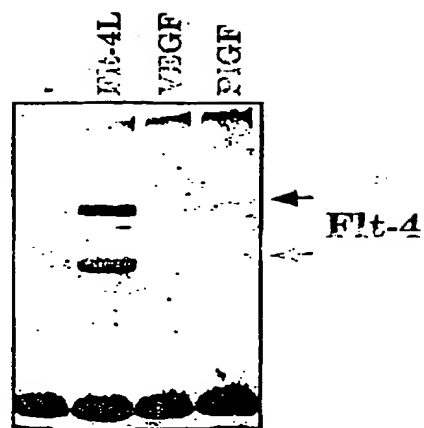
08/510133

FIGURE 7



08/51013

FIGURE 8



08/510131

MetThrValLeuTyrProGluTyr
GAGCAGTTACCGTCTCTCTCCAGTGTAGATGAACTCATGACTGTACTCTACCCAGAATAT
10 30 50
TrpLysMetTyrLysCysGlnLeuArgLysGlyGlyTrpGlnHisAsnArgGluGlnAla
TGGAAATGTACAAGTGTACAGCTAAGGAAAGGAGGCTGGCAACATAACAGAGAACAGGCC
70 90 110
AsnLeuAsnSerArgThrGluGluThrIleLysPheAlaAlaAlaHisTyrAsnThrGlu
AACCTCAACTCAAGGACAGAGAGACTATAAAATTTGCTGCAGCACATTATAATACAGAG
130 150 170
IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys
ATCTTGAAAAGTATTGATAATGAGTGGAGAAAGACTCAATGCATGCCACGGGAGGTGTGT
190 210 230
IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProProCysVal
ATAGATGTCCGAAGGAGTTTGGAGTCCGCGACAAACACCTTCTTTAAACCTCCATGTGTG
250 270 290
SerValTyrArgCysGlyGlyCysCysAsnSerGluGlyLeuGlnCysMetAsnThrSer
TCCGTCTACAGATGTCCCGGTGCTGCAATAGTGAGGGGCTGCAGTGCATGAACACCAGC
310 330 350
ThrSerTyrLeuSerLysThrLeuPheGluIleThrValProLeuSerGlnGlyProLys
ACGAGCTACCTCAGCAAGACGTTATTTCAAATTACAGTGCCTCTCTCAAGGCCCAAA
370 390 410
ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal
CCAGTAACAATCAGTTTTCGCAATCACACTTCCTGCCGATGCATGCTCTAAACTGGATGTT
430 450 470
TyrArgGlnValHisSerIleIleArgArgSerLeuProAlaThrLeuProGlnCysGln
TACAGACAAAGTTTATTCCATTATTAGACGTTCCCTGCCAGCAACACTACCACAGTGTGAC
490 510 530
AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu
GCAGCGAACAAAGACCTGCCCAACCAATTACATGTGGAATAATCACATCTGCAGATGCCTG
550 570 590
AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspAspSerThrAspGlyPheHis
CCTCAGCAAGATTTTATGTTTTCCTCGGATGCTGAGATGACTCAACAGATGGATTCCAT
610 630 650
AspIleCysGlyProAsnLysGluLeuAspGluGluThrCysGlnCysValCysArgAla
GACATCTGTGGACCAACAAGGAGCTGGATGAAGAGACCTGTCACTGTGCTCTGCAGAGCG
670 690 710
GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys
GGGCTTCGGCTGCCAGCTGTGGACCCCAAGAACTAGACAGAACTCATGCCAGTGT
730 750 770
ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAsn
GTCTGTAAAAACAACTCTTCCCAGCCAATGTGGGGCAACCGAGAATTTGATCAAAAC
790 810 830
ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys
ACATGCCAGTGTGTATGTAAAGAACCCTGCCCCAGAAATCAACCCCTAAATCTGGAAAA
850 870 890
CysAlaCysGluCysThrGluSerProGlnLysCysLeuLeuLysGlyLysLysPheHis
TGTGCCCTGTGAATGTACAGAAAGTCCACAGAAATGCTTGTAAAAAGGAAAGAGTTCCAC
910 930 950
HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro
CACCACACATCCAGCTGTTACAGACGGCCATGTACGAACCGCCAGAGGCTTGTGAGCCA
970 990 1010
GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln
GGATTTCATATAGTCAACAAGTGTGCTGTTGCTCCCTTCATATTGGAAAAAGACCACAA
1030 1050 1070
MetSerEnd
ATGAGCTAAGATGCTACTGTTTTCGACTTATGATTTTCTATTATGCAAAACTGTGTG
1090 1110 1130

FIGURE 9

08/51013

	1		50
PDGF-A	.MRTWACLLL LGGCYLAHAL AEEAEIPREL IERLARSQIH SIRDLORLLE		
PDGF-B	MNRCWA.LFL SLCCYLRLVS AEGDPIPEEL YEMLSDSHSIR SFDDLQRLH		
PIGFMP VMRLFPFCFLQ LLAGLAL...		
VEGFMNFLLSWVH WSLALLLYLH		
FLT4-LMTVLYPEYWK MYKQLRKGG		
	51		100
PDGF-A	IDSUGAEDAL ETSLEAHGSH AINHVPKRP VPIRRKRSI.EEAIP		
PDGF-B	GDP.GEEDGA ELDLNMTRSH SGGELES... .LARGRRSLG SLTIAEPAMI		
PIGF	PAVPPQOWAL SA..... GNGSSEVEVV P.FQEVWG..R		
VEGF	HAKWSQAAPM AE..... GGGQNHHEVV K.FMDVYQ..R		
FLT4-L	WOHNREQANL NSRTEETIKF AAAHYNTEIL KSIDNEW..K		
	101		150
PDGF-A	AVCKTRTVIY EIPRSQVDPT SANFLIWHPC VEVKRCIGCC NTSSVKQPS		
PDGF-B	AECKTRTEVF EISRLIDRT NANFLVWHPC VEVORCSGCC NNRNVQCRPT		
PIGF	SYCRALERLV DVVSEY..PS EVEHMFSPSE VSLLRCTIGCC GDENLHCVPV		
VEGF	SYCHPIETLV DIFQEY..PD EIEYIFKPC VPLMRCISGCC NDEGLECVPT		
FLT4-L	TCMPREVC I DVGKEF..GV ATNTFFKPC VSVYRCIGCC NSEGLQCMNT		
	151		200
PDGF-A	RVHHRSVKVA KVEYVRKKPK LKEVQVRLEE HLEPA.... AT.....		
PDGF-B	QVQLRPVQVR KIEIVRKKPI FKATVTLED HLAACK.... ETVAARPVT		
PIGF	ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRCECRPLR EKMKPERC..		
VEGF	EESNITMQIM RIKPH..QQG .HIGEMSFLQ HNKCECRPKK DRARQENP..		
FLT4-L	STSYLSKTLF EITVPLSQGP .KPVITISFAN HPSORMSKL DVYRQVHSII		
	201		250
PDGF-A	..SNLNPDRH EEETDVR... ..GKHKFKKTH DKTALKETLG		
PDGF-B	RSPGGSQEQR AKTPQTRVTI RTVVRVRPPK		
PIGFGDAVPRR...		
VEGFCGPCSERRKH LFVQDPQTCK CSCKNTDSRC KARQLELNER		
FLT4-L	RRSLPATLPQ CQAANKTCPT NYMWNHICR CLAQEDFMFS SDAGDDSTDG		
	251		300
PDGF-A		
PDGF-B	A.....		
PIGF		
VEGF	TCRCDKPRR..		
FLT4-L	FHDICGPKE LDEETQCVC RAGLRPASCG PHKELDRNSC QCVCKNKLFP		
	301		350
PDGF-A		
PDGF-B		
PIGF		
VEGF		
FLT4-L	SQCGANREFD ENTQCVCCKR TCRNQPLNP GKCAECTES PQKCLLKGGK		
	351		395
PDGF-A		
PDGF-B		
PIGF		
VEGF		
FLT4-L	FHHOTCSCYR RPCTNRQKAC EPGFSYSEEV CRCVPSYWKR PQMS		

FIGURE 10

08/510133

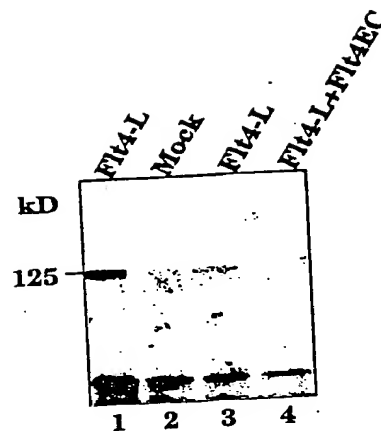


FIGURE 11

08/510131

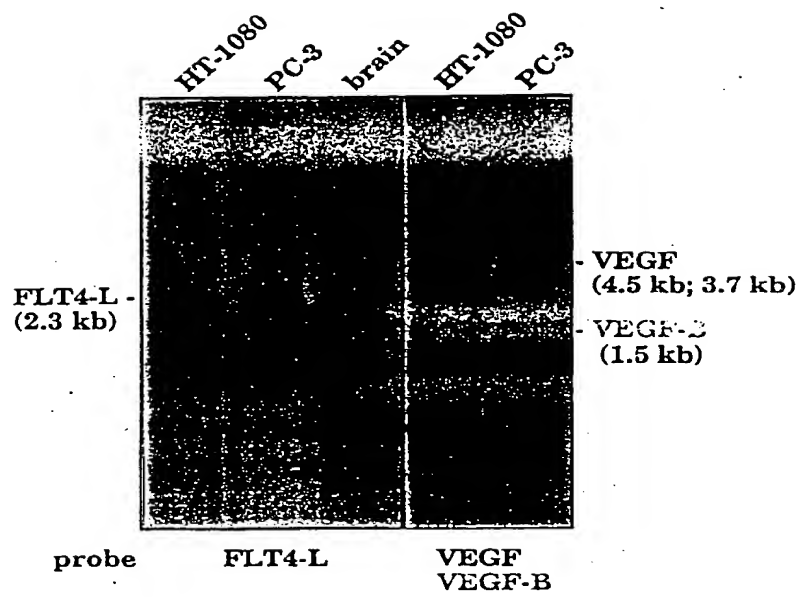


FIGURE 12

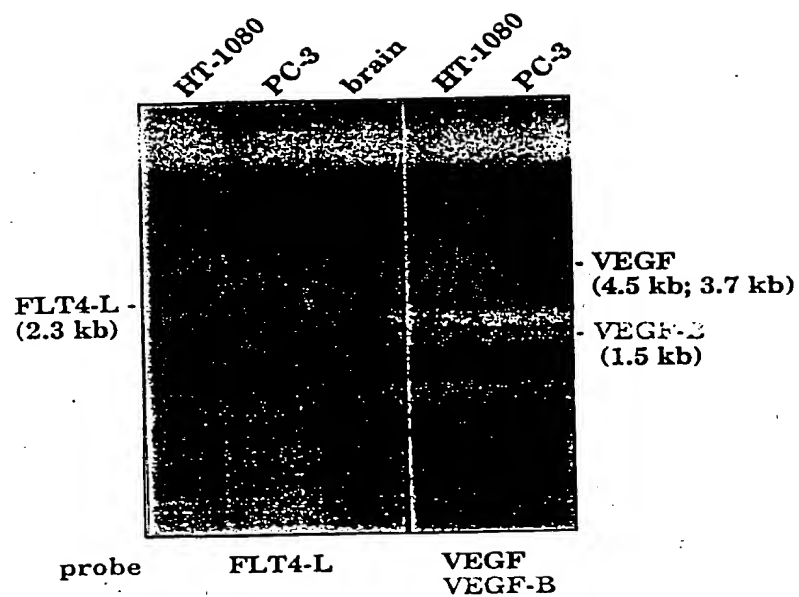


FIGURE 12



08/510133

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No: 28113/32863

PATENT APPLICATION TRANSMITTAL

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Kari Alitalo and Vladomir Joukov

Title: "Receptor Ligand"

1. Type of Application

This new application is for a

- ☒ utility patent.
☐ design patent.

2. Application Papers Enclosed

- 1 Title Page
40 Pages of Specification (excluding Claims, Abstract & Drawings)
2 Pages of Claims
1 Page of Abstract
12 Sheets of Drawings (Figs. 1 to 12)
☐ Formal
☒ Informal

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on August 1, 1995, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EG473138672US.


Thomas C. Meyers

3. Declaration or Oath

- ☐ Enclosed
- ☐ Executed by (check all applicable boxes)
- ☐ Inventor(s)
- ☐ Legal representative of inventor(s)
(37 CFR 1.42 or 1.43)
- ☐ Joint inventor or person showing a proprietary interest on behalf of
inventor who refused to sign or cannot be reached
- ☐ The petition required by 37 CFR 1.47 and the statement required
by 37 CFR 1.47 are enclosed. See Item 5D below for fee.
- ☒ Not enclosed - the undersigned attorney or agent is authorized to file this
application on behalf of the applicant(s). An executed declaration will follow.

4. Additional Papers Enclosed

- ☐ Preliminary Amendment
- ☐ Information Disclosure Statement
- ☐ Declaration of Biological Deposit
- ☒ Computer readable copy of sequence listing containing nucleotide and/or amino
acid sequence
- ☐ Verified statement(s) claiming small entity status under 37 CFR 1.9 and 1.27
- ☐ Associate Power of Attorney
- ☐ Verified translation of a non-English patent application
- ☐ An assignment of the invention
- ☐ Certified copy(ies) of application(s):

COUNTRY	APPLICATION NO.	FILED

from which priority under 35 USC 119 is claimed ☐ is(are) attached.

☐ will follow.

☐ Other

5. Filing Fee Calculation (37 CFR 1.16)

A. ☒ Utility Application

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	NO. FILED	NO. EXTRA	RATE	FEE	RATE	FEE
BASIC FEE				\$365.00		\$730.00
TOTAL	12 - 20	= 0	X 11 =	\$	X 22 =	\$
INDEP.	3 - 3	= 0	X 38 =	\$	X 76 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 120 =	\$	+ 240 =	
Filing Fee:				\$	OR	\$730.00

B. ☐ Design Application (\$150.00/\$300.00)

Filing Fee: \$ _____

C. ☐ Plant Application (\$245.00/\$490.00)

Filing Fee: \$ _____

D. Other Fees

☐ Recording Assignment [Fee -- \$40.00 per assignment] \$ _____

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached [Fee -- \$130.00] \$ _____

☐ Other \$ _____

Total Fees Enclosed \$730.00

6. Method of Payment of Fees

☒ Check in the amount of: \$730.00

☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

☐ Not enclosed

7. Deposit Account and Refund Authorization

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 or under other applicable rules (except payment of issue fees), to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Please direct all future communications to Thomas C. Meyers, at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 

Thomas C. Meyers
Reg. No: 36,989

August 1, 1995

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

Atty. Docket No: 28113/32863

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which (check one): ☐ is attached hereto; ☒ was filed on August 1, 1995 as Application Serial No. 08/510,133 and was amended on _____ (if applicable); ☐ was filed as PCT International Application No. _____ on _____ and was amended under Article 19 on _____ (if applicable). I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

(Application Serial Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

(Application Serial Number)	(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements are the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shulman (19,412)	Trevor B. Joike (25,542)	Richard A. Schnurr (39,890)	James J. Napoli (32,361)
Donald J. Brott (19,490)	Timothy J. Vezeau (26,348)	Anthony Nimmo (30,920)	Richard M. La Barge (32,254)
Owen J. Murray (22,111)	Carl E. Moore, Jr. (26,487)	Christine A. Dudzik (31,245)	Jeffrey W. Smith (33,455)
Allen H. Gerstein (22,218)	Richard H. Anderson (26,526)	Kevin D. Hogg (31,839)	Douglass C. Hochstetler (33,710)
Nate F. Scarpelli (22,320)	Patrick D. Ertel (26,877)	Jeffrey S. Sharp (31,879)	Cynthia L. Schaller (34,245)
Edward M. O'Toole (22,477)	James P. Zeller (28,491)	Donald J. Pochopien (32,167)	Robert M. Gerstein (34,824)
Michael F. Borun (25,447)	William E. McCracken (30,195)	Martin J. Hirsch (32,237)	

Send correspondence to: Thomas C. Meyers

FIRM NAME	PHONE NO.	STREET	CITY & STATE	ZIP CODE
Marshall, O'Toole, Gerstein, Murray & Borun	312-474-6300	6300 Sears Tower 233 South Wacker Drive	Chicago, Illinois	60606-6402

Full Name of First or Sole Inventor Kari Alitalo	Citizenship Finland
Residence Address - Street Nyyrikintie 4A	Post Office Address - Street Same
City (Zip) 02100 Espoo	City (Zip) Same
State or Country FINLAND	State or Country Same
Date November 30, 1995	Signature [Signature]

See second page for additional inventor(s)

See reverse for relevant rules & statutes

APPLICABLE RULES AND STATUTES

37 CFR 1.56. DUTY OF DISCLOSURE: INFORMATION MATERIAL TO PATENTABILITY (Applicable Portion)

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentability defines, to make sure that any material information contained therein is disclosed to the Office.

Information relating to the following factual situations enumerated in 35 USC 102 and 103 may be considered material under 37 CFR 1.56(a).

35 U.S.C. 102. CONDITIONS FOR PATENTABILITY: NOVELTY AND LOSS OF RIGHT TO PATENT

A person shall be entitled to a patent unless --

- (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent, or
- (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States, or
- (c) he has abandoned the invention, or
- (d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months before the filing of the application in the United States, or
- (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraph (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or
- (f) he did not himself invent the subject matter sought to be patented, or
- (g) before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other.

35 U.S.C. 103. CONDITIONS FOR PATENTABILITY: NON-OBVIOUS SUBJECT MATTER

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negative by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

35 U.S.C. 112. SPECIFICATION (Applicable Portion)

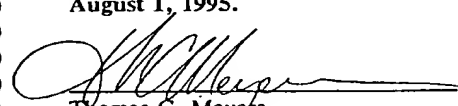
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Second Joint Inventor, if any Vladimir Joukov 2-100	Citizenship Finland
Residence Address - Street Topeliuksenkatu 32G8	Post Office Address - Street Same
City (Zip) 00290 Helsinki FK	City (Zip) Same
State or Country FINLAND	State or Country Same
Date 30.11.1995	Signature V. Joukov

08/51013

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:)	I hereby certify that this paper is
)	being deposited with the United
Alitalo, <i>et al.</i>)	States Postal Service, in an envelope
)	addressed to: Assistant
Serial No.: TBD)	Commissioner for Patents,
)	Washington, DC 20231 utilizing the
)	"Express Mail Post Office to
Filed: Herewith)	Addressee" service of the United
)	States Postal Service under Mailing
)	Label No. EG 473 138 672 US on
For: "Receptor Ligand")	August 1, 1995.
)	
)	
)	Thomas C. Meyers
)	Reg. No. 36,989
)	Attorney for Applicants

TRANSMITTAL OF SEQUENCE LISTING AND
STATEMENT UNDER 37 C.F.R. §1.821(f)

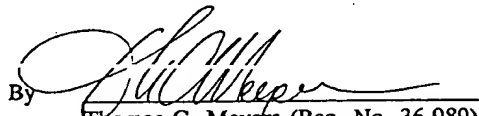
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I hereby state that the content of the paper and computer readable
copies of the Sequence Listing, submitted in accordance with 37 C.F.R. §1.821(c)
and (e), respectively, are the same.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By 
Thomas C. Meyers (Reg. No. 36,989)
Attorneys for Applicants
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

TEAM 8

PAGE: 1

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/510,133

DATE: 10/04/95
TIME: 11:46:31

INPUT SET: S6494.raw

This Raw Listing contains the General
Information Section and up to the first 5 pages.

SEQUENCE LISTING

(1) General Information:

(i) APPLICANT: Alitalo, Kari
Joukov, Vladomir

(ii) TITLE OF INVENTION: Receptor Ligand

(iii) NUMBER OF SEQUENCES: 33

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
(B) STREET: 6300 Sears Tower, 233 South Wacker Drive
(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606-6402

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Meyers, Thomas C.
(B) REGISTRATION NUMBER: 36,989
(C) REFERENCE/DOCKET NUMBER: 28113/32863

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312/474-6300
(B) TELEFAX: 312/474-0448
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

ENTERED

PAGE: 2

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/510,133

DATE: 10/04/95
TIME: 11:46:34

INPUT SET: S6494.raw

```

47         (D) TOPOLOGY: linear
48
49         (ii) MOLECULE TYPE: DNA (genomic)
50
51         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
52
53         TGTCCTCGCT GTCCTTGTCT                                20
54
55         (2) INFORMATION FOR SEQ ID NO:2:
56
57         (i) SEQUENCE CHARACTERISTICS:
58             (A) LENGTH: 70 base pairs
59             (B) TYPE: nucleic acid
60             (C) STRANDEDNESS: single
61             (D) TOPOLOGY: linear
62
63         (ii) MOLECULE TYPE: DNA (genomic)
64
65         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
66
67         ACATGCATGC CACCATGCAG CGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG        60
68
69         GACTCCTGGA                                            70
70
71         (2) INFORMATION FOR SEQ ID NO:3:
72
73         (i) SEQUENCE CHARACTERISTICS:
74             (A) LENGTH: 24 base pairs
75             (B) TYPE: nucleic acid
76             (C) STRANDEDNESS: single
77             (D) TOPOLOGY: linear
78
79         (ii) MOLECULE TYPE: DNA (genomic)
80
81         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
82
83         ACATGCATGC CCCGCCGGTC ATCC                                24
84
85         (2) INFORMATION FOR SEQ ID NO:4:
86
87         (i) SEQUENCE CHARACTERISTICS:
88             (A) LENGTH: 22 base pairs
89             (B) TYPE: nucleic acid
90             (C) STRANDEDNESS: single
91             (D) TOPOLOGY: linear
92
93         (ii) MOLECULE TYPE: DNA (genomic)
94
95         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
96
97         CGGAATTCCC CATGACCCCA AC                                22
98
99         (2) INFORMATION FOR SEQ ID NO:5:
```

PAGE: 3

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/510,133

DATE: 10/04/95
TIME: 11:46:37

INPUT SET: S6494.raw

```
100
101      (i) SEQUENCE CHARACTERISTICS:
102          (A) LENGTH: 33 base pairs
103          (B) TYPE: nucleic acid
104          (C) STRANDEDNESS: single
105          (D) TOPOLOGY: linear
106
107      (ii) MOLECULE TYPE: DNA (genomic)
108
109      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
110
111      CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT 33
112
113      (2) INFORMATION FOR SEQ ID NO:6:
114
115          (i) SEQUENCE CHARACTERISTICS:
116              (A) LENGTH: 17 base pairs
117              (B) TYPE: nucleic acid
118              (C) STRANDEDNESS: single
119              (D) TOPOLOGY: linear
120
121          (ii) MOLECULE TYPE: DNA (genomic)
122
123          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
124
125          ATTTAGGTGA CACTATA 17
126
127      (2) INFORMATION FOR SEQ ID NO:7:
128
129          (i) SEQUENCE CHARACTERISTICS:
130              (A) LENGTH: 34 base pairs
131              (B) TYPE: nucleic acid
132              (C) STRANDEDNESS: single
133              (D) TOPOLOGY: linear
134
135          (ii) MOLECULE TYPE: DNA (genomic)
136
137          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
138
139          CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT 34
140
141      (2) INFORMATION FOR SEQ ID NO:8:
142
143          (i) SEQUENCE CHARACTERISTICS:
144              (A) LENGTH: 40 amino acids
145              (B) TYPE: amino acid
146              (C) STRANDEDNESS: single
147              (D) TOPOLOGY: linear
148
149          (ii) MOLECULE TYPE: protein
150
151          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
152
```

PAGE: 4

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/510,133

DATE: 10/04/95
TIME: 11:46:40

INPUT SET: S6494.raw

```
153 Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
154 1 5 10 15
155
156 Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
157 20 25 30
158
159 His Arg Gln Glu Ser Gly Phe Arg
160 35 40
161
```

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGAGTCGA CTTGGCGGAC T

21

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC

60

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC

34

(2) INFORMATION FOR SEQ ID NO:12:

PAGE: 5

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/510,133

DATE: 10/04/95
TIME: 11:46:44

INPUT SET: S6494.raw

206 (1) SEQUENCE CHARACTERISTICS:
207 (A) LENGTH: 20 base pairs
208 (B) TYPE: nucleic acid
209 (C) STRANDEDNESS: single
210 (D) TOPOLOGY: linear
211
212 (ii) MOLECULE TYPE: DNA (genomic)
213
214 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

215 GTTGCCTGTG ATGTGCACCA

20

217
218 (2) INFORMATION FOR SEQ ID NO:13:
219
220 (i) SEQUENCE CHARACTERISTICS:
221 (A) LENGTH: 18 amino acids
222 (B) TYPE: amino acid
223 (C) STRANDEDNESS: single
224 (D) TOPOLOGY: linear
225
226 (ii) MOLECULE TYPE: peptide
227
228 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

229
230 Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
231 1 5 10 15
232
233 Leu Lys
234
235

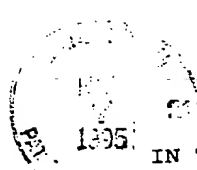
236 (2) INFORMATION FOR SEQ ID NO:14:
237
238 (i) SEQUENCE CHARACTERISTICS:
239 (A) LENGTH: 17 base pairs
240 (B) TYPE: nucleic acid
241 (C) STRANDEDNESS: single
242 (D) TOPOLOGY: linear
243

244 (ii) MOLECULE TYPE: DNA (genomic)
245
246 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

247
248 GCAGARGARA CNATHAA

17

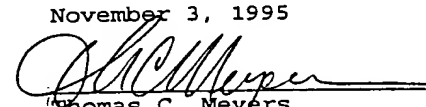
249
250 (2) INFORMATION FOR SEQ ID NO:15:
251
252 (i) SEQUENCE CHARACTERISTICS:
253 (A) LENGTH: 5 amino acids
254 (B) TYPE: amino acid
255 (C) STRANDEDNESS: single
256 (D) TOPOLOGY: linear
257
258 (ii) MOLECULE TYPE: DNA (genomic)



6300

Handwritten: #6 MW 51-90
PATENT 0300

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:)	I hereby certify that this
)	paper is being deposited
Alitalo, et al.)	with the United States
)	Postal Service as first
Serial No.: 08/510,133)	class mail, postage
)	prepaid, in an envelope
)	addressed to:
Filed: August 1, 1995)	Commissioner of Patents
)	and Trademarks,
For: "Receptor Ligand")	Washington, DC 20231 on
)	this date:
)	
Group Art Unit: TBD)	November 3, 1995
)	
Examiner: TBD)	
)	Thomas C. Meyers
)	Reg. No. 36,989
)	Attorney for Applicants

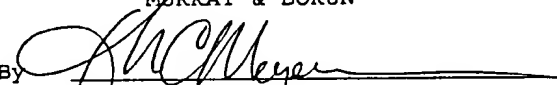
INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the attached form PTO-1449 and the items of information cited therein are hereby submitted by Applicants for consideration in connection with the above-identified patent application. This statement and the accompanying items of information, including form PTO-1449, are being submitted within three months of the filing of the above-identified patent application. Accordingly, it is submitted that no fee is due in this matter under 37 C.F.R. §1.97(b). However, if it is determined that any appropriate fee is due, please charge Deposit Account No. 13-2855. A duplicate of this paper is enclosed.

Respectfully submitted,
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By 
Thomas C. Meyers
Reg. No. 36,989
Attorneys for Applicants
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

November 3, 1995

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28113/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo, <i>et al.</i>	
		Filing Date 8/1/95	Group

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
PKL	C1	Ausprunk, <i>et al.</i> , "Migration and Proliferation of Endothelial Cells in Preformed and Newly Formed Blood Vessels during Tumor Angiogenesis", <i>Microvascular Research</i> , 14:53-65 (1977).
PKL	C2	Breier, <i>et al.</i> , "Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation", <i>Development</i> , 114:521-532 (1992).
PKL	C3	Dignam, <i>et al.</i> , "Balbiani ring 3 in <i>Chironomus tentans</i> encodes a 185-kDa secretory protein which is synthesized throughout the fourth larval instar", <i>Gene</i> , 88:133-140 (1990).
PKL	C4	Don, <i>et al.</i> , "'Touchdown' PCR to circumvent spurious priming during gene amplification", <i>Nucleic Acids Research</i> , 19(14):4008 (1991).
PKL	C5	Folkman, <i>et al.</i> , "Angiogenesis", <i>The Journal of Biological Chemistry</i> , 267(16):10931-10934 (1992).
PKL	C6	Kozak, "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs", <i>Nucleic Acids Research</i> , 15(20):8125-8148 (1987).
PKL	C7	Mäkelä, <i>et al.</i> , "Plasmid pLTRpoly: A Versatile High-Efficiency Mammalian Expression Vector", <i>Gene</i> , 118:293-294 (1992).
PKL	C8	Pajusola, <i>et al.</i> , "FLT4 Receptor Tyrosine Kinase Contains Seven Immunoglobulin-like Loops and Is Expressed in Multiple Human Tissues and Cell Lines", <i>Cancer Research</i> , 52:5738-5743 (1992).
PKL	C9	Pajusola, <i>et al.</i> , "Two human FLT4 receptor tyrosine kinase isoforms with distinct carboxy terminal tails are produced by alternative processing of primary transcripts", <i>Oncogene</i> , 8:2931-2937 (1993).
PKL	C10	Risau, <i>et al.</i> , "Changes in the Vascular Extracellular Matrix during Embryonic Vasculogenesis and Angiogenesis", <i>Developmental Biology</i> , 125:441-450 (1988).
PKL	C11	Saksela, <i>et al.</i> , "Cell-Associated Plasminogen Activation: Regulation and Physiological Functions", <i>Annu. Rev. Cell. Biol.</i> , 4:93-126 (1988).
PKL	C12	Tessier, <i>et al.</i> , "Enhanced secretion from insect cells of a foreign protein fused to the honeybee melittin signal peptide", <i>Gene</i> , 98:177-183 (1991).
PKL	C13	van der Geer, <i>et al.</i> , "Receptor Protein-Tyrosine Kinases and Their Signal Transduction Pathways", <i>Annu. Rev. Cell. Biol.</i> , 10:251-337 (1994).

EXAMINER Erin Lohr	DATE CONSIDERED 8/28/96
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28113/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo, et al.	
		Filing Date 8/1/95	Group

20

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
BKL	C1	Ausprunk, et al., "Migration and Proliferation of Endothelial Cells in Preformed and Newly Formed Blood Vessels during Tumor Angiogenesis", <i>Microvascular Research</i> , 14:53-65 (1977).
BKL	C2	Breier, et al., "Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation", <i>Development</i> , 114:521-532 (1992).
BKL	C3	Dignam, et al., "Babiani ring 3 in <i>Chironomus tentans</i> encodes a 185-kDa secretory protein which is synthesized throughout the fourth larval instar", <i>Gene</i> , 88:133-140 (1990).
BKL	C4	Don, et al., "'Touchdown' PCR to circumvent spurious priming during gene amplification", <i>Nucleic Acids Research</i> , 19(14):4008 (1991).
BKL	C5	Folkman, et al., "Angiogenesis", <i>The Journal of Biological Chemistry</i> , 267(15):10931-10934 (1992).
BKL	C6	Kozak, "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs", <i>Nucleic Acids Research</i> , 15(20):8125-8148 (1987).
BKL	C7	Mäkelä, et al., "Plasmid pLTRpoly: A Versatile High-Efficiency Mammalian Expression Vector", <i>Gene</i> , 118:293-294 (1992).
BKL	C8	Pajusola, et al., "FLT4 Receptor Tyrosine Kinase Contains Seven Immunoglobulin-like Loops and Is Expressed in Multiple Human Tissues and Cell Lines", <i>Cancer Research</i> , 52:5738-5743 (1992).
BKL	C9	Pajusola, et al., "Two human FLT4 receptor tyrosine kinase isoforms with distinct carboxy terminal tails are produced by alternative processing of primary transcripts", <i>Oncogene</i> , 8:2931-2937 (1993).
BKL	C10	Risau, et al., "Changes in the Vascular Extracellular Matrix during Embryonic Vasculogenesis and Angiogenesis", <i>Developmental Biology</i> , 125:441-450 (1988).
BKL	C11	Saksela, et al., "Cell-Associated Plasminogen Activation: Regulation and Physiological Functions", <i>Annu. Rev. Cell. Biol.</i> , 4:93-126 (1988).
BKL	C12	Tessier, et al., "Enhanced secretion from insect cells of a foreign protein fused to the honeybee melittin signal peptide", <i>Gene</i> , 98:177-183 (1991).
BKL	C13	van der Geer, et al., "Receptor Protein-Tyrosine Kinases and Their Signal Transduction Pathways", <i>Annu. Rev. Cell. Biol.</i> , 10:251-337 (1994).

EXAMINER Eric M. Luthy	DATE CONSIDERED 8/28/96
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	



136-105-AA
UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
--------------------	-------------	-----------------------	------------------------

136-105-AA 09/20/05 136-105-AA 2011070560

STEWART, JOHN E. GERSTEIN
JERRY AND JOYCE
2110 N. 10TH AVE
300 SOUTH WACKER DRIVE
CHICAGO, IL 60606-6100

028271120

DATE MAILED: 09/06

**NOTICE TO FILE MISSING PARTS OF APPLICATION
FILING DATE GRANTED**

11/20/05

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 130 for large entities or \$ 65 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 130.

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit \$ _____ to complete the basic filing fee.
2. ☐ Additional claim fees of \$ _____ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
3. ☒ The oath or declaration:
☒ is missing.
☐ does not cover the newly submitted items.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.

4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
5. ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:
_____. An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ _____ under 37 CFR 1.17(k), unless this fee has already been paid.
8. ☐ A \$ _____ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).
9. ☐ Your filing receipt was mailed in error because your check was returned without payment.
10. ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with Sequence Rules 37 CFR 1.821-1.825.
11. ☐ Other: 09/20/05 1 105 130.00 CK

Direct the response to Box Missing Part and refer any questions to the Customer Service Center at (703) 308-1202.

A copy of this notice MUST be returned with the response.



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
--------------------	-------------	-----------------------	------------------------

03/510,133 08/01/95 ALITALO

K 28113/32863

0282/1120

MARSHALL O'TOOLE GERSTEIN
MURRAY AND BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

DATE MAILED: 0000

**NOTICE TO FILE MISSING PARTS OF APPLICATION
FILING DATE GRANTED**

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 130 for large entities or \$ 65 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 130.

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit \$ _____ to complete the basic filing fee.
2. ☐ Additional claim fees of \$ _____ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
3. ☒ The oath or declaration:
☒ is missing.
☐ does not cover the newly submitted items.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.
4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
5. ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:
_____. An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ _____ under 37 CFR 1.17(k), unless this fee has already been paid.
8. ☐ A \$ _____ processing fee is required since your check was returned without payment (37 CFR 1.21(m)).
9. ☐ Your filing receipt was mailed in error because your check was returned without payment.
10. ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with Sequence Rules 37 CFR 1.821-1.825.
11. ☐ Other.

Direct the response to Box Missing Part and refer any questions to the Customer Service Center at (703) 308-1202.

A copy of this notice MUST be returned with the response.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:)	
Kari Alitalo and Vladomir Joukov)	Title: Receptor Ligand
Serial No: 08/510,133)	
Filed: August 1, 1995)	

TRANSMITTAL OF EXECUTED DECLARATION

***Assistant Commissioner for Patents
Washington, D.C. 20231***

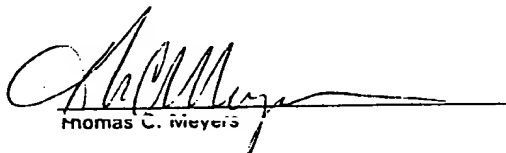
Attention: Application Branch

Sir:

Submitted herewith is an executed Declaration for filing in the above-identified application, in response to the Notice to File Missing Parts issued by the Patent and Trademark Office on November 20, 1995.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on December 19, 1995 in an envelope addressed to Assistant Commissioner for Patents, Washington, D.C. 20231.


Thomas C. Meyers

Also enclosed is a copy of the Notice together with our check in the amount of \$130.00 in payment of the fee.

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this request is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 

Thomas C. Meyers
Reg. No: 36,989

December 19, 1995




1-115 Group

1814

#8781
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):)	<u>CERTIFICATE OF MAILING</u>
)	<u>(37 CFR 1.8)</u>
Alitalo et al.)	
Serial No: 08/510,133)	I hereby certify that this paper and the
Filed: August 1, 1995)	documents referred to as enclosed therewith
Title: Receptor Ligand)	are being deposited with the United States
Group Art Unit: 1814)	Postal Service as first class mail, postage
Examiner: Lathrop, B.)	prepaid, on July 24, 1996, in an envelope
)	addressed to the Assistant Commissioner for
)	Patents, Washington, D.C. 20231.
)	
)	David A. Gass

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants hereby petition pursuant to 37 CFR 1.136(a) for a one month extension of time. Attached is a check in the amount of \$110.00 in payment of the petition fee.

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 to Deposit Account No. 13-2855. A copy of this Petition is enclosed.

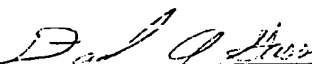
Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: 1-1-97

By:


David A. Gass
Reg. No: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Serial Number: 08/510,133
Art Unit: 1814

-3-


7. A telephone call was made to David Gass on 16 May 1996 to request an oral election to the above restriction, but the call did not result in an election being made.

8. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop whose telephone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

The examiner will attempt to respond to voice mail messages within 24 hours. Alternately, the examiners's supervisor, Robert A. Wax, can be reached at (703) 308-4216. The FAX number for Group 1814 is (703) 305-7401.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Brian K. Lathrop, Ph.D.
AU 1814


ROBERT A. WAX
SUPERVISORY PATENT EXAMINER
GROUP 180

Serial Number: 08/510,133
Art Unit: 1814

-2-

Part III DETAILED ACTION

Election/Restriction

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:
Group I. Claims 1, 2, 8-10, and 12, drawn to an Flt4 ligand and a pharmaceutical composition comprising the same, classified in Class 514, subclass 2.
Group II. Claims 3-7, drawn to nucleic acid encoding an Flt4 ligand, classified in Class 536, subclass 23.5.
Group III. Claim 11, drawn to an antibody reactive against an Flt4 ligand, classified in Class 530, subclass 387.9.

The inventions are distinct, each from the other, because of the following reasons:

2. The protein of Group I is a patentably distinct chemical species from the nucleic acid of Group II, although related as the nucleic acid encodes the Flt4 ligand. The ligand can be made without recourse to the nucleic acid by standard methods of biochemical purification from tissues, and the nucleic acid has separate utility as a probe for detection of complementary genomic sequences, for example.
3. The protein of Group I is a patentably distinct chemical species from the antibody of Group III, although the antibody is directed to the Flt4 ligand. The ligand can be made without recourse to the antibody by standard methods of biochemical purification from tissues, and the antibody has a separate utility as a probe for screening an expression library, for example.
4. The antibody of Group III is a patentably distinct chemical species from the nucleic acid of Group II, because the antibody can be raised to ligand purified without recourse to the nucleic acid, and the nucleic acid has separate utility as a probe for detection of complementary genomic sequences, for example.
5. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, and because the search for each group of inventions is not coextensive with the searches of the inventions of the other groups, restriction for examination purposes as indicated is proper.
6. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
---------------	-------------	----------------------	---------------------

1812/0329 ALIYANU

28113/32863

EXAMINER

LAFAROF, E

ART UNIT PAPER NUMBER

1812/0329

GERSTEIN MURRAY

2000 PEARSON TOWER

200 SOUTH WACKER DRIVE

CHICAGO IL 60606-6402

1814

DATE MAILED:

05/29/96

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☐ Responsive to communication filed on _____ ☐ This action is made final.

Authority: statutory period for response to this action is set to expire _____ month(s), 30 days from the date of this letter.
Failure to respond within this period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I: THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- | | |
|---|---|
| 1. <input type="checkbox"/> Notice of References Cited by Examiner, PTO-892. | 2. <input type="checkbox"/> Notice of Draftsman's Patent Drawing Review, PTO-948. |
| 3. <input type="checkbox"/> Notice of Art Cited by Applicant, PTO-1449. | 4. <input type="checkbox"/> Notice of Informal Patent Application, PTO-152. |
| 5. <input type="checkbox"/> Information on how to Effect Drawing Changes, PTO-1474. | 6. <input type="checkbox"/> _____ |

Part II: SUMMARY OF ACTION

1. ☒ Claims 1-12 are pending in the application.
Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☐ Claims _____ are rejected.
5. ☐ Claims _____ are objected to.
6. ☒ Claims 1-12 are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____, has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

III. The Applicants Elect Claims 1, 2, 8-10, and 12 (Group I) with traverse.

In response to the restriction requirement, the Applicants hereby elect Group I (claims 1, 2, 8-10, and 12), with traverse.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: July 24, 1996

By:

David A. Gass
David A. Gass
Reg. No: 38,153

inventions.") No such burden has been alleged in the Office action.¹ In fact, removal of the restriction requirement will conserve the resources of the Patent and Trademark Office and the Applicants, by minimizing the number of similar searches performed by the Patent Office examiners and by reducing the filing fees and prosecution costs of the Applicants.

For example, the Patent Office has acknowledged a relationship between a protein and the nucleic acids which encode it. When conducting a thorough prior art search for the purified and isolated polypeptide of the invention (Group I), the Patent Office will undoubtedly wish to search the art related to nucleic acids (Group II) which encode proteins, due to the universal nature of the genetic code. Moreover, because it is a common practice in the art to report nucleic acid and deduced amino acid sequences simultaneously, these arts are highly co-extensive. The burden on the Patent Office in searching both the nucleic acid and amino acid arts, if any, will not be serious.

Similarly, it is a common practice in the art to characterize antibodies with reference to the antigen which the antibodies recognize. Therefore, when conducting a thorough prior art search related to the antibody claim 11 (Group III), the Patent Office will undoubtedly search the art related to proteins, i.e., the art that it will search for the claims of Group I.

Thus, a thorough examination of the claims in any particular group will involve a search of the art pertinent to the subject matter of the claims of the other groups. Hence, no serious burden will result if restriction is not required in this case. However, if the restriction requirement is maintained, the Applicants will incur additional prosecution costs associated with filing and prosecuting divisional applications, and the Patent and Trademark Office will be required to perform a duplicative search of the same prior art. Thus, withdrawal of the restriction requirement will conserve the resources of the Patent and Trademark Office and of the Applicants without causing a serious burden on the Patent and Trademark Office.

For these reasons, the restriction requirement imposed in the Office action should be withdrawn.

¹ The different Patent and Trademark Office classifications of the claims in Groups I, II, and III are not an indication of independence or distinction under §121. Nor are the classifications an indication that an undue burden exists on the Examiner. Such classifications merely serve as a convenient search tool.

design, operation or effect" See M.P.E.P. §802.01 (emphasis added). In the restriction requirement, the Patent Office has acknowledged that the allegedly distinct three groups of claims are related. For example, the Patent Office states that the "protein of Group I" and "the nucleic acid of Group II" are "related as the nucleic acid encodes the Flt4 ligand." (Restriction Requirement at p. 2.) Thus, a nucleic acid which encodes a protein is connected in operation and effect to the encoded protein. The lack of "independence" between Groups I and II also is manifest from the claims themselves. For example, claims 3-7 of Group II depend from claim 2 of Group I. Because the claims of Groups I and II are related, the subject matters of the two groups are not "independent" as required by 35 U.S.C. §121, and the restriction requirement was improper as between these two groups of claims.

Similarly, the claim of Group III, "drawn to an antibody," is not "independent" of the claims of Group I, "drawn to an Flt4 ligand." For example, an antibody to a protein is connected to the protein in design, operation, and effect because one skilled in the art uses the protein (or portions thereof) as an antigen to generate the antibody. The antibody, in turn, reacts immunologically with the protein with a great degree of specificity. Because the claims of Groups I and III are related, the groups are not "independent."

For the reasons stated above, the Examiner has not demonstrated that the divided Groups of claims are "independent" from each other as required by 35 U.S.C. §121. Thus, the Examiner has failed to meet the burden for imposing a restriction requirement.

- II. The restriction requirement should be withdrawn because the Examiner has not demonstrated a serious burden will result if restriction is not required, and because withdrawal will conserve resources of the Patent and Trademark Office and the Applicants.

Assuming *arguendo* that the claims as grouped by the Examiner are "independent" and "distinct," the restriction requirement is nonetheless improper, because the Examiner has failed to demonstrate that "a serious burden" will result if restriction is not required. See M.P.E.P. §803 ("If the search and examination of an entire application can be made without serious burden, the examiner must examine it on the merits, even though it includes claims to distinct or independent



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No. 08/510,133

Filed: August 1, 1995

For: RECEPTOR LIGAND

Art Unit: 1814

Examiner: Lathrop, B.

) I hereby certify that this paper is
) being deposited with the United
) States Postal Service as first class
) mail, postage prepaid, in an
) envelope addressed to: Assistant
) Commissioner for Patents
) Washington, D.C. 20231, on this
) date:

) Dated: July 24, 1996

) David A. Gass
) David A. Gass

ELECTION WITH TRAVERSE IN RESPONSE TO RESTRICTION REQUIREMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In an official communication dated May 29, 1996, the U.S. Patent and Trademark Office issued a restriction requirement in the above-identified patent application, and set a 30 day period for response. This response to the restriction requirement has been timely filed with a petition for one month extension of time and petition fee. Reconsideration of the restriction requirement is respectfully requested in light of the following remarks.

- I. The restriction requirement should be withdrawn because the Examiner has not demonstrated that the "independent and distinct" provisions of 35 U.S.C. §121 have been satisfied.

The Examiner has required restriction of the claims of the application for examination purposes, citing 35 U.S.C. §121. The Applicants traverse the restriction requirement. Reconsideration is requested.

The provisions of 35 U.S.C. §121 state, "If two or more independent and distinct inventions are claimed in one application, the Commissioner may require the application to be restricted to one of the inventions." (Emphasis added.) The Examiner has failed to demonstrate or assert that the claims divided into Groups I, II, and III are "independent" from each other.

According to the Patent Office's own procedure manual, "The term 'independent' (i.e., not dependent) means that there is n o d i s c l o s e d r e l a t i o n s h i p between the two or more subjects disclosed, that is, they are unconnected in

AUG 12 1996 12:34PM

MARSHALL O'TOOLE

No. 6103 P. 2/5
From: 0808

FAX CENTER
RECEIVED

AUG 12 1996

OFFICIAL

GROUP 1800

PATENT
28113/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.

Serial No: 08/510,133

Filed: August 1, 1995

Title: RECEPTOR LIGAND

Group Art Unit: 1814

Examiner: Lathrop, B.

I hereby certify that this paper is
being deposited with the United
States Postal Service with sufficient
postage as first class mail in an
envelope addressed to: Assistant
Commissioner for Patents,
Washington, D.C., 20231 on this
date:

Date: August 12, 1996

David A. Gass
David A. Gass
Registration No. 38,153
Attorney for Applicants

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

The Applicants respectfully request entry of this Preliminary
Amendment prior to examination of the above-identified application on the merits
by the Patent and Trademark Office.

AMENDMENTS

In the Specification:

At page 1, line 2, please insert the following priority claim:

-- This application is a continuation-in-part of U.S. Patent Application Serial No.
08/340,011, filed November 14, 1994. --

At page 26, line 10, please delete "pFLT4" and substitute therefor --
pFlt4-L--.

At page 26, line 12, please delete "_____" and substitute
therefor --97231--.

In the claims:

Please amend claims 1-4, 6, 8-9, and 12, and add new claims 13-19
as shown below:

1. (Amended) A purified and isolated polypeptide [peptide] which
specifically binds to the Flt4 receptor tyrosine kinase.

2. (Amended) A purified and isolated polypeptide comprising an
[peptide having the] amino acid sequence shown in SEQ ID NO: 33.

3. (Amended) A purified and isolated nucleic acid encoding the
purified and isolated polypeptide [peptide] according to claim 2.

4. (Amended) The nucleic acid according to claim 3 having [the]
a nucleotide sequence shown in SEQ ID NO: 32.

6. (Amended) The vector according to claim 5, wherein said vector is
plasmid [pFlt4] pFlt4-L, deposited as ATCC accession No. 97231.

8. (Amended) A fragment of the purified and isolated [peptide]
polypeptide according to claim 2 which is capable of specifically binding to an Flt4
receptor tyrosine kinase.

9. (Amended) The fragment according to claim 8 having an apparent
molecular weight of approximately 23 kD as assessed by SDS-PAGE under
reducing conditions.

12. (Amended) A pharmaceutical composition comprising a [peptide] polypeptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

13. A polypeptide according to claim 1 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

14. A polypeptide according to claim 13 which is capable of stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

15. A purified and isolated polypeptide according to claim 14, said polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13.

16. A purified and isolated polypeptide according to claim 13, wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

17. A purified and isolated polypeptide according to claim 1, said polypeptide being purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435.

18. A conditioned medium comprising a polypeptide according to claim 1.

19. A polypeptide according to claim 1 further comprising a detectable label. --

REMARKS

The specification has been amended herein to claim priority from an earlier-filed U.S. application. This amendment is accompanied by a supplemental inventors' declaration which acknowledges this priority claim.

The specification has been amended at page 26 to make reference to a Budapest Treaty biological deposit of a vector of the invention that is described at p. 26, lines 5-8. Such an amendment does not constitute new matter. See *In re Lundak*, 773 F.2d 1216, 1223, 227 U.S.P.Q. 90, 96 (Fed. Cir. 1985). Claim 6 has been similarly amended to claim this vector.

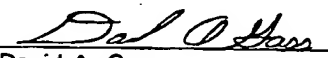
The amendment to claim 9 finds support throughout the specification, including at p. 18, lines 26-30.

New claims 13-18 find support throughout the specification, including in Example 5, and particularly at p. 18, line 16, to p. 19, line 19.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Dated: August 12, 1996


David A. Gass
Registration No. 38,153
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
--------------------	-------------	-----------------------	---------------------

FILE NO.

K 28113/32863

EXAMINER

1. What is the purpose of the study?

287 2/0910

GENSTEIN SUPPLY

100

1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2029, 2030, 2031, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077, 2078, 2079, 2080, 2081, 2082, 2083, 2084, 2085, 2086, 2087, 2088, 2089, 2090, 2091, 2092, 2093, 2094, 2095, 2096, 2097, 2098, 2099, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260, 2261, 2262, 2263, 2264, 2265, 2266, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313, 2314, 2315, 2316, 2317, 2318, 2319, 2320, 2321, 2322, 2323, 2324, 2325, 2326, 2327, 2328, 2329, 2330, 2331, 2332, 2333, 2334, 2335, 2336, 2337, 2338, 2339, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2347, 2348, 2349, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2368, 2369, 2370, 2371, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2381, 2382, 2383, 2384, 2385, 2386, 2387, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 2399, 2400, 2401, 2402, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 2440, 2441, 2442, 2443, 2444, 2445, 2446, 2447, 2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2456, 2457, 2458, 2459, 2460, 2461, 2462, 2463, 2464, 2465, 2466, 2467, 2468, 2469, 2470, 2471, 2472, 2473, 2474, 2475, 2476, 2477, 2478, 2479, 2480, 2481, 2482, 2483, 2484, 2485, 2486, 2487, 2488, 2489, 2490, 2491, 2492, 2493, 2494, 2495, 2496, 2497, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506, 2507, 2508, 2509, 2510, 2511, 2512, 2513, 2514, 2515, 2516, 2517, 2518, 2519, 2520, 2521, 2522, 2523, 2524, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2533, 2534, 2535, 2536, 2537, 2538, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2548, 2549, 2550, 2551, 2552, 2553, 2554, 2555, 2556, 2557, 2558, 2559, 2560, 2561, 2562, 2563, 2564, 2565, 2566, 2567, 2568, 2569, 2570, 2571, 2572, 2573, 2574, 2575, 2576, 2577, 2578, 2579, 2580, 2581, 2582, 2583, 2584, 2585, 2586, 2587, 2588, 2589, 2590, 2591, 2592, 2593, 2594, 2595, 2596, 2597, 2598, 2599, 2600, 2601, 2602, 2603, 2604, 2605, 2606, 2607, 2608, 2609, 2610, 2611, 2612, 2613, 2614, 2615, 2616, 2617, 2618, 2619, 2620, 2621, 2622, 2623, 2624, 2625, 2626, 2627, 2628, 2629, 2630, 2631, 2632, 2633, 2634, 2635, 2636, 2637, 2638, 2639, 2640, 2641, 2642, 2643, 2644, 2645, 2646, 2647, 2648, 2649, 2650, 2651, 2652, 2653, 2654, 2655, 2656, 2657, 2658, 2659, 2660, 2661, 2662, 2663, 2664, 2665, 2666, 2667, 2668, 2669, 2670, 2671, 2672, 2673, 2674, 2675, 2676, 2677, 2678, 26

DATE: 46 MAY 1981 VZ

09-6492

ART UNIT

PAPER NUMBER

ii)

1814

DATE MAILED: 181

09/10/95

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

- ☒ Responsive to communication(s) filed on ~~5/1/96~~ 7/26/96
- ☐ This action is **FINAL**.
- ☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.**

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

- ☒ Claim(s) 1-19 is/are pending in the application.
- Of the above, claim(s) 3-7, 11 is/are withdrawn from consideration.
- ☒ Claim(s) 2, 12 is/are allowed.
- ☒ Claim(s) 1, 8-10, 13-19 is/are rejected.
- ☒ Claim(s) 14, 17 is/are objected to.
- ☐ Claims are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been
☐ received.
- ☐ received in Application No. (Series Code/Serial Number) _____
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received:

- [] Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☐ Notice of Reference Cited, PTO-892
- ☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 5
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Notice of Informal Patent Application, PTO-152

- SEE OFFICE ACTION ON THE FOLLOWING PAGES -

Serial Number: 08/510133
Art Unit: 1814

2

Part III DETAILED ACTION

Election/Restriction

I. Applicant's election with traverse of Group I (claims 1, 2, 8-10, and 12) in Paper No. 9 is acknowledged. The traversal is on the grounds that (1) the Restriction Requirement has not demonstrated that the inventions satisfy the "independent and distinct" provision of 35 U.S.C. § 121, (2) the Restriction Requirement has not demonstrated burden, and (3) withdrawal of the requirement for restriction will conserve resources of the Office and applicant. This is not found persuasive because of the following reasons:

(1) This Action respectfully refers to M.P.E.P. § 802.01, which states:

The law has long been established that dependent inventions . . . may be properly divided if they are, in fact, "distinct" inventions, even though dependent.

Further elaboration of "distinct" is provided in M.P.E.P. § 802.01. The Requirement for Restriction has correctly defined the distinctness of the inventions as required by 35 U.S.C. § 121 for the reasons made of record in Paper No. 9. Restriction would be improper if prior art reading on one invention would make the other obvious. Prior art anticipating a protein does not necessarily render the encoding DNA obvious because of the redundancy of the genetic code (M.P.E.P. § 2144.09, p. 104). Prior art anticipating a protein does not necessarily make the antibody obvious, because motivation must be present in the prior art to obtain that antibody (M.P.E.P. § 2143.01), and because antibodies may be of different varieties, which has raised different issues regarding obviousness in the case law.

(2) In reference to M.P.E.P. § 803, this Action respectfully refers to M.P.E.P. § 808.02, wherein it states that *prima facie* burden is correctly established by demonstration of separate classification. A thorough search of the relevant prior art in both electronic databases and in the

Serial Number: 08/510133
Art Unit: 1814

3

patent files is fundamental in establishing patentability and underlies this *prima facie* showing of burden. Prior art searches on proteins, nucleic acids, or antibodies must address separate issues of patentability in each case:

(3) This Action, while cognizant of the financial resources expended on prosecution, respectfully submits that consideration of monetary burden on Office or applicant is not recognized as a ground for establishing a burden to examine or a lack thereof.

The requirement is still deemed proper and is therefore made FINAL.

2. Claims 3, 4, 6 (each amended), 5, 7, and 11 are withdrawn from further consideration by the examiner, 37 C.F.R. § 1.142(b), as being drawn to non-elected inventions, the requirement having been traversed in Paper No. 9.
3. This application contains claims 3, 4, 6 (each amended), 5, 7, and 11 drawn to inventions non-elected with traverse in Paper No. 9. A complete response to the final rejection must include cancellation of non-elected claims or other appropriate action (37 C.F.R. § 1.144; M.P.E.P. § 821.01).
4. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).
5. Pursuant to the Preliminary Amendment in Paper No. 10, claims 1 (amended), 2 (amended), 8 (amended), 9 (amended), 10, 12 (amended), and newly added claims 13-19 are pending in the instant application.

Serial Number: 08/510133
Art Unit: 1814

4

Priority

6. If the filing date of an application to which priority is claimed under 35 U.S.C. § 120 is needed to overcome an intervening reference, there is a need for the Office to make a determination as to whether the requirement of 35 U.S.C. § 120, that the earlier application discloses the invention of the second application in the manner provided by the first paragraph of 35 U.S.C. 112, is met and whether a substantial portion of all of the earlier application is repeated in the second application in a continuation-in-part situation (M.P.E.P. § 201.08).

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. § 112, first paragraph, have been clarified in *Ex parte Forman*, 230 USPQ 546 (BPAI 1986). As summarized in *Ex parte Forman*, the following considerations are relevant to the analysis of enablement for the claimed invention of an Flt4 ligand in application Serial No. 08/340011. First, the instant specification teaches at p.7, line38 through p.8, line 1, that the Flt receptor *may* prove useful in obtaining a ligand that binds to it, but no such method is taught. There are no other teachings of the ligand. Second, no working examples are given that teach a ligand to the receptor. Third, the skilled artisan at the time of the instant invention had no means of predicting which compounds would be ligands for the receptor, the requisite degree of affinity for specific binding to the receptor was unknown and could not be predicted, and success in using the receptor to obtain the ligand could not be predicted. Additionally, the specification does not teach that the expressed receptor is functionally active, and the skilled artisan cannot predict the success of any method of obtaining a ligand to a receptor that is not biologically active. Thus, undue experimentation would be required to make the instant ligand. Consequently, no claims are afforded priority to application Serial No. 08/340011.

Serial Number: 08/510133
Art Unit: 1814

5

Oath/Declaration

7. The oath or declaration is defective. A new oath or declaration in compliance with 37 C.F.R. § 1.67(a) identifying this application by its Serial Number and filing date is required. See M.P.E.P. §§ 602.01 and 602.02.

The oath or declaration is defective because:

It does not state that the person making the oath or declaration in a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, acknowledges the duty to disclose material information as defined in 37 C.F.R. § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

The Examiner notes that the supplemental declaration was to be included in Paper No. 10, but was either misplaced or omitted during transmittal.

Drawings

8. This application has been filed with informal drawings which are acceptable for examination purposes only. Pursuant to a change in Office policy effective 25 April 1996, *formal drawings will be required at the time allowable subject matter is first indicated.*

Please note the Examiner incorrectly submitted the PTO 948 to the Draftman for review. Review will be appropriate upon submission of formal drawings; the PTO 948 will not be attached to the Office action in Paper No. 11.

Specification

9. The disclosure is objected to because of the following informalities: 3' deletions of the Flt4 ligand are taught at p. 27, line 24, but deletions occurring at the end of a protein are preferably termed "C-terminal" or "carboxy terminal" deletions. "3'" is preferred for describing nucleic acids only. Appropriate correction is required.

10. The lengthy specification has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicant's cooperation is requested in correcting any errors of which applicant may become aware in the specification.

Serial Number: 08/510133
Art Unit: 1814

6

Claim Objections

11. Claim 17 is objected to under 37 C.F.R. § 1.75(c) for not further limiting the subject matter of claim 1.

Specifically, the instant claim does not recite further structural limitations to the polypeptide of claim 1; the particular source of the polypeptide is *de minimus*.

12. Claim 14 is objected to under 37 C.F.R. § 1.75(c) for not further limiting the subject matter of claim 13.

Claim 14 recites the functional limitation of stimulating Flt4 phosphorylation, which is a functional limitation not enabled by the teachings of the specification (M.P.E.P. § 2173.05(g)). The specification teaches a polypeptide of 23 kD that stimulates Flt4 phosphorylation at Figure 5. The specification does not teach necessary or sufficient structures of the 23 kD protein that promote phosphorylation, nor could the skilled artisan have predicted what these structures would be from the state of the art at the time of the invention. Claim 14 does not further limit claim 13, because the limitation of promoting phosphorylation in the instant claim does not further limit the structure of the polypeptide of claim 13.

Claim Rejections - 35 USC § 112

13. Claims 1 and 8, and dependent claims 9, 10, 13-19 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The term "specifically" in claims 1 and 8 is a relative term which renders the claim indefinite. The term "specifically" is not defined by the claim, the specification does not provide a

Serial Number: 08/510133
Art Unit: 1814

7

standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

The art can define high affinity and non-specific binding to receptors; however, the instant inventions encompasses numerous polypeptides which would be expected to have affinities for the receptor anywhere between high affinity to non-specific binding. Intermediate affinities are not definable without a standard of affinity for "specific" binding, and hence it is impossible to determine whether such compounds would be included within the bounds of the claims.

14. Claim 10 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The claim is presumably drawn to a polypeptide but refers to SEQ ID NO:32, which is a nucleic acid sequence.

Furthermore, the term "approximately" in claim 10 is a relative term which renders the claim indefinite. The term "approximately" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

In the instant case, it is not clear whether "approximately" is used in reference to the length or the composition of the polypeptide. In both cases the bounds of the claim cannot be determined without a standard of reference. It is not clear, for example, whether the claim encompasses polypeptides larger than the first 180 amino acids such as the precursor to the protein of SEQ ID NO:32.

Serial Number: 08/510133
Art Unit: 1814

8

15. Claims 8-10 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, fragments of a polypeptide comprising SEQ ID NO:33 are claimed. As such, the fragments must consist of *at least* the amino acids of SEQ ID NO:33. It is unclear whether this is the intended meaning, because the fragments of claims 9 and 10, for example, appear to encompass only part of SEQ ID NO:33.

16. Claims 1, 13-15 and dependent claims 17-19 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for the scope of claim 16, does not reasonably enable the range of polypeptides encompassed in the instant claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. § 112, first paragraph, have been clarified in *Ex parte Forman*, 230 USPQ 546 (BPAI 1986). As summarized in *Ex parte Forman*, the following considerations are relevant to the analysis of enablement for the instantly claimed invention. First, the specification teaches at Example 5 a purified polypeptide having an apparent molecular weight of approximately 23 kD and an N-terminal sequence consisting of SEQ ID NO:13. No other polypeptides which specifically bind the Flt4 receptor are taught. Second, claim 1 encompasses all proteins that may interact with the Flt4 receptor, and claim 13 encompasses all 23 kD fragments of any portion of all of these polypeptides. Claim 15 encompasses all 23 kD polypeptides comprising the 17 amino acids of SEQ ID NO:13 which may be present at any region of these polypeptides. This claim encompasses about 34 polypeptides from the protein of

Serial Number: 08/510133
Art Unit: 1814

9

SEQ ID NO:33, because the position of SEQ ID NO:13 relative to the N-terminal residue is not specified. Third, the skilled artisan would predict that some proteins which would bind the Flt4 receptor would be growth factors. It was known that many different growth factors were found in PC-3 conditioned medium as taught by Sitaras et al., for example. A vast number of additional polypeptides might interact with the receptor, even if the interaction was relatively weak. The skilled artisan could not predict which of this vast number of polypeptides, or 23 kD fragments thereof, would bind the Flt4 receptor or stimulate its tyrosine kinase activity, because the secondary structures required for interacting with the receptor were unknown and the structures of most of the proteins that may interact with the receptor were unknown. As Ferrara et al. teach at column 29, lines 29-32, even endothelial growth factors of the same size may have widely different structures and potencies. Thus, the skilled artisan would not predict that any protein characterized to no other extent than having an apparent molecular weight of 23 kD would bind the receptor. Fourth, a vast amount of experimentation would be required to make and test all the encompassed polypeptides of the instant invention. Fifth, the skilled artisan could not predict which of the approximately 34 polypeptides of claim 15 would bind the receptor, because these polypeptides would have different secondary structures determined by their different compositions, and the structures required for interacting with the receptor were unknown. For the reasons set forth above, an undue amount of experimentation would be required to make the claimed invention.

Serial Number: 08/510133
Art Unit: 1814

10

17. Claims 8 and 9 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for the scope of claim 10, does not reasonably enable the range of polypeptides encompassed in the instant claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

As summarized in *Ex parte Forman*, the following considerations are relevant to the analysis of enablement for the instantly claimed invention. First, claim 8 encompasses fragments of all sizes from all locations of the protein of SEQ ID NO:33, and claim 9 encompasses all of these same fragments having an apparent molecular weight of 23 kD. Second, the specification teaches at Example 5 a purified polypeptide having an apparent molecular weight of approximately 23 kD and an N-terminal sequence consisting of SEQ ID NO:13. The specification also teaches at Example 11 that a fragment having amino acids 1-180 of SEQ ID NO:33 *may* bind the receptor. Third, the skilled artisan could not predict which of the vast number of polypeptides encompassed by the claims would bind the Flt4 receptor, because the secondary structures required for interacting with the receptor were unknown and the secondary structures of any of the fragments of the claimed polypeptide were unknown. As Ferrara et al. teach at column 29, lines 29-32, even endothelial growth factors of the same size may have widely different structures and potencies. Thus, the skilled artisan would not predict that any fragment of the protein comprising SEQ ID NO:33 would bind the receptor solely because it's 23 kD in size. Fourth, a vast amount of experimentation would be required to make all the encompassed fragments and test their ability to bind the receptor. Thus, an undue amount of experimentation would be required to make and use the claimed invention.

Serial Number: 08/510133
Art Unit: 1814

11

Claim Rejections - 35 USC § 102

18. The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

19. Claim 1 is rejected under 35 U.S.C. § 102(a) as being anticipated by Pajusola et al.

Pajusola et al. teach at p. 3550, column 1, a purified polypeptide, colony stimulating factor-1 (CSF-1), which binds a CSF-1 receptor/Flt4 fusion protein. An inherent property of the fusion protein is the Flt4 receptor tyrosine kinase. Pajusola et al. thus anticipate claim 1.

20. Claim 18 is rejected under 35 U.S.C. § 102(b) as being anticipated by Sitaras et al.

Sitaras et al., the whole document, teach a conditioned medium, thus anticipating the claimed invention. Because this conditioned medium is from PC-3 prostatic adenocarcinoma cells, it has the inherent property of comprising the polypeptide of claim 1.

Serial Number: 08/510133
Art Unit: 1814

12

Allowable Subject Matter

21. Claims 2 and 12 are allowable over the prior art of record.
22. The following is an Examiner's statement of reasons for the indication of allowable subject matter:

Tischer, et al. motivate the discovery of vascular endothelial growth factors at Background of the Invention for the advantageous result of pharmaceutical compositions for promoting wound healing. Galland et al., however, teach at p. 1238, column 2, that the physiological role of the Flt4 receptor was unknown at the time of the invention, complicating the search for a specific ligand. Fournier et al., herein cited as evidence of the state of the art at the time of the invention, further teach at p. 921, column 2, that a specific ligand for Flt4 receptor had not been identified at the time of the invention and that known vascular endothelial growth factors did not specifically bind Flt4 receptor. Human Genome Sciences, Inc. published the sequence of a Flt4 receptor ligand with greater than 99 percent identity to the instant ligand, but the date of publication does not antecede the filing date of the instant application. The ligand of Human Genome Sciences, Inc was purified using expressed sequence tags without identification as encoding a vascular endothelial growth factor; therefore, one of ordinary skill in the art at the time of the invention would not have been motivated to use these art-known sequences to arrive at the instant invention.

Serial Number: 08/510133
Art Unit: 1814

13

23. Claims 8-10 would be allowable if rewritten to overcome the rejection under 35 U.S.C. § 112 and to include all of the limitations of the base claim and any intervening claims.


Conclusion

24. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop whose telephone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

The examiner will attempt to respond to voice mail messages within 24 hours. Alternately, the examiner's supervisor, Robert A. Wax, can be reached at (703) 308-4216. The FAX number for Group 1814 is (703) 305-7401.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Brian K. Lathrop, Ph.D.
AU 1814


ROBERT A. WAX
SUPERVISORY PATENT EXAMINER
GROUP 180

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

1 of 2

FORM PTO-892 (REV. 2-92)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NO. 08/510133	GROUP/ART UNIT 1814	ATTACHMENT TO PAPER NUMBER 11			
NOTICE OF REFERENCES CITED				APPLICANT(S) Alitalo et al.					
U.S. PATENT DOCUMENTS									
•		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE		
	A	5337671	6/26/94	Ferrara et al.	435	240.1			
	B	5219739	6/15/93	Tischer et al.	435	69.4			
	C								
	D								
	E								
	F								
	G								
	H								
	I								
	J								
	K								
FOREIGN PATENT DOCUMENTS									
•		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG.	PP. SPEC.
	L	9524473	9/14/95	WO	Human Genome Sciences, Inc.	—	—		
	M								
	N								
	O								
	P								
	Q								
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)									
	R	Sitaras et al. Constitutive production of platelet-derived growth factor-like protein by human prostate carcinoma cell lines. Cancer Research. Vol. 48, No. 7, pages 1730-1735. 4/1/88							
	T	Fournier et al. Mutation at tyrosine residue 1337 abrogates ligand-dependent transforming capacity of the FLT4 receptor. Oncogene. Vol. 11, No. 5, pages 921- 931. 9/7/95							
EXAMINER		DATE							
Brian Lathrop		8/30/96							
* A copy of this reference is not being furnished with this office action. (See Manual of Patent Examining Procedure, section 707.05 (a).)									

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

2 of 2

FORM PTO-892 (REV. 2-92)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NO. 08/510 133	GROUP/ART UNIT 1814	ATTACHMENT TO PAPER NUMBER 11			
NOTICE OF REFERENCES CITED 2				APPLICANT(S) Alitalo et al.					
U.S. PATENT DOCUMENTS									
		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE		
A									
B									
C									
D									
E									
F									
G									
H									
I									
J									
K									
FOREIGN PATENT DOCUMENTS									
		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG.	PP. SPEC.
L									
M									
N									
O									
P									
Q									
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)									
R		Rajavasa et al. Signalling properties of FLT4, a proteolytically processed receptor tyrosine kinase related to two VEGF receptors. Oncogene. Vol. 9, No. 12, pages 3545-3555. 12/94							
T		Galland et al. The FLT4 gene encodes a transmembrane tyrosine kinase related to the vascular endothelial growth factor receptor. Oncogene. Vol. 8, No. 5, pages 1233-1240. 5/93							
EXAMINER Brian Lathrop			DATE 8/30/96						
* A copy of this reference is not being furnished with this office action. (See Manual of Patent Examining Procedure, section 707.05 (a).)									

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 08/01/95	Group 1814

U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate

FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No
B1	WO 96/11269 A2	04/18/96	PCT	C12N	15/12		
B2	WO 95/33050 A1	12/07/95	PCT	C12N	15/12		
B3	WO 96/30046 A1	10/03/96	PCT	A61K	39/395		
B4	WO 96/39421 A1	12/12/96	PCT	C07H	21/04		
B5	WO 96/39515 A1	12/12/96	PCT	C12N	15/12		

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

C14	Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors and Receptors Involved in Angiogenesis," <i>The 9th International Conference of the International Society of Differentiation (ISD), Development Cell Differentiation and Cancer</i> , Pisa (Italy), September 28-October 2, 1996, p. 66 (ABSTRACT S22).
C15	Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors B and C Receptors Involved in Angiogenesis," <i>German-American Academic Council Foundation(GAAC)/Stiftung Deutsch-Amerikanisches Akademisches Konzil (DAAK), 2nd Symposium on Current Problems in Molecular Medicine: The Role of Cytokines in Human Disease</i> , November 17-20, 1996, Ringberg Castle, Germany, p. 1 (ABSTRACT).

EXAMINER <i>[Signature]</i>	DATE CONSIDERED <i>8/5/97</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 08/01/95	Group 1814

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

C16	Andersson <i>et al.</i> , "Assignment of Interchain Disulfide Bonds in platelet-Derived Growth Factor (PDGF) and Evidence for Agonist Activity of Monomeric PDGF," <i>J. Biol. Chem.</i> , 267(16):11260-11266 (June 5, 1992).
C17	Aprelikova <i>et al.</i> , "FLT4, A Novel Class III Receptor Tyrosine Kinase in Chromosome 5q33-qter," <i>Cancer Research</i> , 52:746-748 (February 1, 1992).
C18	Basilico <i>et al.</i> , "The FGF Family of Growth Factors and Oncogenes," <i>Adv. Cancer Res.</i> , 59:145-165 (1992).
C19	Berse <i>et al.</i> , "Vascular Permeability Factor (Vascular Endothelial Growth Factor) Gene is Expressed Differentially in Normal Tissues, Macrophages, and Tumors," <i>Mol. Biol. Cell.</i> , 3:211-220 (February, 1992).
C20	Betsholtz <i>et al.</i> , "cDNA Sequence and Chromosomal Localization of Human Platelet-Derived Growth Factor A-Chain and Its Expression in Tumor Cell Lines," <i>Nature</i> , 320:695-699 (April, 1986).
C21	Borg <i>et al.</i> , "Biochemical Characterization of Two Isoforms of FLT4, a VEGF Receptor-Related Tyrosine Kinase," <i>Oncogene</i> , 10:973-84 (1995).
C22	Cao <i>et al.</i> , "Heterodimers of Placenta Growth Factor/Vascular Endothelial Growth Factor," <i>J. Biol. Chem.</i> , 271(6):3154-3162 (February 9, 1996).
C23	Cheng and Flanagan, "Identification and Cloning of ELF-1, A Developmentally Expressed Ligand for the Mek4 and Sek Receptor Tyrosine Kinases," <i>Cell</i> , 79:157-168 (October 7, 1994).
C24	Claesson-Welsh <i>et al.</i> , "Identification and Structural Analysis of the A Type Receptor for Platelet-derived Growth Factor," <i>J. Biol. Chem.</i> , 264(3):1742-1747 (January 25, 1989).
C25	Coffin <i>et al.</i> , "Angioblast Differentiation and Morphogenesis of the Vascular Endothelium in the Mouse Embryo," <i>Devel. Biol.</i> , 148:51-62 (1991).
C26	Curran and Franza, "Fos and Jun: The AP-1 Connection," <i>Cell</i> , 55:395-397 (November 4, 1988).
C27	De Vries <i>et al.</i> , "The <i>fms</i> -Like Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor," <i>Science</i> , 255:989-991 (February 21, 1992).

EXAMINER <i>[Signature]</i>	DATE CONSIDERED <i>4/3/97</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 08/01/95	Group 1814

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

C28	DiSalvo <i>et al.</i> , "Purification and Characterization of a Naturally Occurring Vascular Endothelial Growth Factor: Placenta Growth Factor Heterodimer," <i>J. Biol. Chem.</i> , 270(13):7717-7723 (March 31, 1995).
C29	Dumont <i>et al.</i> , "Dominant-negative and Targeted Null Mutations in the Endothelial Receptor Tyrosine Kinase, <i>tek</i> , Reveal a Critical Role in Vasculogenesis of the Embryo," <i>Genes Dev.</i> , 8:1897-1909 (1994).
C30	Dumont <i>et al.</i> , "Vascularization of the Mouse Embryo: A Study of <i>flk-1</i> , <i>tek</i> , <i>tie</i> , and Vascular Endothelial Growth Factor Expression During Development," <i>Development Dynamics</i> , 203:80-92 (1995).
C31	Dvorak <i>et al.</i> , "Review: Vascular Permeability Factor/Vascular Endothelial Growth Factor, Microvascular Hyperpermeability, and Angiogenesis," <i>Amer. J. Path.</i> , 146:1029-1039 (1995).
C32	Eichmann <i>et al.</i> , "Two Molecules Related to the VEGF Receptor are Expressed in Early Endothelial Cells During Avian Embryonic Development," <i>Mech. Dev.</i> , 42:33-48 (1993).
C33	Ferrara <i>et al.</i> , "Molecular and Biological Properties of the Vascular Endothelial Growth Factor Family of Proteins," <i>Endocrine Rev.</i> , 13(1):18-32 (1992).
C34	Finnerty <i>et al.</i> , "Molecular Cloning of Murine FLT and FLT4," <i>Oncogene</i> , 8(11):2293-2298 (1993).
C35	Flamme <i>et al.</i> , "Vascular Endothelial Growth Factor (VEGF) and VEGF-Receptor 2 (<i>flk-1</i>) are Expressed During Vasculogenesis and Vascular Differentiation in the Quail Embryo," <i>Devel. Biol.</i> , 169:699-712 (1995).
C36	Flanagan and Leder, "The <i>kit</i> Ligand: A Cell Surface Molecule Altered in Steel Mutant Fibroblasts," <i>Cell</i> , 63:185-194 (October 5, 1990).
C37	Folkman, "Angiogenesis in Cancer, Vascular, Rheumatoid and Other Disease," <i>Nature Med.</i> , 1(1):27-31 (1995).
C38	Folkman <i>et al.</i> , "Long-term Culture of Capillary Endothelial Cells," <i>Proc. Nat'l Acad. Sci., USA</i> , 76(10):5217-5221 (October, 1979).

EXAMINER <i>3/1/97</i>	DATE CONSIDERED <i>4/1/97</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 08/01/95	Group 1814

12

MAIL ROOM
FEB 13 1997

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

C39	Fong <i>et al.</i> , "Role of the Flt-1 Receptor Tyrosine Kinase in Regulating the Assembly of Vascular Endothelium," <i>Nature</i> , 376:66-70 (July 6, 1995).
C40	Friesel <i>et al.</i> , "Molecular Mechanisms of Angiogenesis: Fibroblast Growth Factor Signal Transduction," <i>FASEB J.</i> , 9:919-25 (1995).
C41	Genbank S66407, "FLT4 Receptor Tyrosine Kinase Isoform FLT4 Long (3' Region, Alternatively Spliced) [Human, mRNA Partial, 216 nt].," Deposited by Pajusola <i>et al.</i> , Dated December 17, 1993.
C42	Genbank U48800, "Mus Musculus Vascular Endothelial Growth Factor B Precursor (VEGF-B) mRNA, Complete Cds.,," Deposited by Olofsson <i>et al.</i> , Dated August 19, 1996.
C43	Genbank X15997, "Human Vascular Permeability Factor mRNA, Complete Cds.,," Deposited by Keck <i>et al.</i> , Dated June 15, 1990.
C44	Genbank X60280, "Vector Plasmid pLTRpoly DNA.,," Deposited by Maekelae, T.P., Dated July 16, 1996.
C45	Genbank X68203, "H. sapiens mRNA for FLT4, Class III Receptor Tyrosine Kinase.,," Deposited by Aprelikova, O., Dated November 30, 1993.
C46	Genbank X94216, "Homo sapiens mRNA for VEGF-C protein," Deposited by Joukov <i>et al.</i> , Dated February 6, 1996.
C47	Harlow <i>et al.</i> , <i>Antibodies, a Laboratory Manual</i> , Cold Spring Harbor Laboratory Press, pp. 72-137, 141-157, 287 & 321-358 (1988).
C48	Heldin <i>et al.</i> , "Structure of Platelet-Derived Growth Factor: Implications for Functional Properties," <i>Growth Factors</i> , 8:245-252 (1993).
C49	Joukov <i>et al.</i> , "A Novel Vascular Endothelial Growth Factor, VEGF-C, Is a Ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) Receptor Tyrosine Kinases," <i>EMBO J.</i> , 15(2):290-298 (1996).
C50	Kaipainen <i>et al.</i> , "Expression of the FMS-Like Tyrosine Kinase 4 Gene Becomes Restricted to Lymphatic Endothelium During Development," <i>Proc. Nat'l Acad. Sci., USA</i> , 92:3566-3570 (April, 1995).

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 08/01/95	Group 1814

13



OTHER DOCUMENTS (Include Author, Title, Date, Pertinent Pages, etc.)		
C51	Kaipainen <i>et al.</i> , "The Related Flt4/PLT1 and KDR Receptor Tyrosine Kinases Show Distinct Expression Patterns in Human Fetal Endothelial Cells," <i>J. Exp. Med.</i> , 178:2077-2088 (December, 1993).	
C52	Kaipainen <i>et al.</i> , "Enhanced Expression of the Tie Receptor Tyrosine Kinase Messenger RNA in the Vascular Endothelium of Metastatic Melanomas," <i>Cancer Res.</i> , 54:6571-6577 (December 15, 1994).	
C53	Kukk <i>et al.</i> , "VEGF-C Receptor Binding and Pattern of Expression with VEGFR-3 Suggests a Role in Lymphatic Vascular Development," <i>Development</i> , 122:3829-3837 (1996).	
C54	Lee <i>et al.</i> , "Vascular Endothelial Growth Factor-Related Protein: A Ligand and Specific Activator of the Tyrosine Kinase Receptor Flt4," <i>Proc. Nat'l Acad. Sci., USA</i> , 93:1988-1992 (March, 1996).	
C55	Leung <i>et al.</i> , "Vascular Endothelial Growth Factor Is a Secreted Angiogenic Mitogen," <i>Science</i> , 246:1306-1309 (December 8, 1989).	
C56	Levy <i>et al.</i> , "Post-transcriptional Regulation of Vascular Endothelial Growth Factor by Hypoxia," <i>J. Biol. Chem.</i> , 271(5):2746-2753 (February 2, 1996).	
C57	Levy <i>et al.</i> , "Transcriptional Regulation of the Rat Vascular Endothelial Growth Factor Gene by Hypoxia," <i>J. Biol. Chem.</i> , 270(22):13333-13340 (June 2, 1995).	
C58	Lyman <i>et al.</i> , "Molecular Cloning of a Ligand for the flt3/ftk-2 Tyrosine Kinase Receptor: A Proliferative Factor for Primitive Hematopoietic Cells," <i>Cell</i> , 75:1157-1167 (December 17, 1993).	
C59	Maglione <i>et al.</i> , "Isolation of a Human Placenta cDNA Coding for a Protein Related to the Vascular Permeability Factor," <i>Proc. Nat'l Acad. Sci., USA</i> , 88:9267-9271 (October, 1991).	
C60	Maglione <i>et al.</i> , "Two Alternative mRNAs Coding for the Angiogenic Factor, Placenta Growth Factor (PIGF) are Transcribed from a Single Gene of Chromosome 14," <i>Oncogene</i> , 8:925-931 (1993).	

EXAMINER <i>[Signature]</i>	DATE CONSIDERED <i>[Signature]</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 08/01/95	Group 1814



OTHER DOCUMENTS (including Author, Title, Date, Pertinent Pages, etc.)		
C61	Matthews <i>et al.</i> , "A Receptor Tyrosine Kinase cDNA Isolated from a Population of Enriched Primitive Hematopoietic Cells and Exhibiting Close Genetic Linkage to c-kit," <i>Proc. Nat'l Acad. Sci., USA</i> , 88:9026-9030 (October, 1991).	
C62	Metzelaar <i>et al.</i> , "CD63 Antigen," <i>J. of Biol. Chem.</i> , 266(5):3239-3245 (February 15, 1991).	
C63	Millauer <i>et al.</i> , "Glioblastoma Growth Inhibited <i>in vivo</i> by a Dominant-Negative Flk-1 Mutant," <i>Nature</i> , 367:576-579 (February 10, 1994).	
C64	Millauer <i>et al.</i> , "High Affinity VEGF Binding and Developmental Expression Suggest Flk-1 as a Major Regulator of Vasculogenesis and Angiogenesis," <i>Cell</i> , 72:835-846 (March 26, 1993).	
C65	Mitchell <i>et al.</i> , "Transcription Factor AP-2 is Expressed in Neural Crest Cell Lineages During Mouse Embryogenesis," <i>Genes and Dev.</i> , 5:105-119 (1991).	
C66	Morgenstern <i>et al.</i> , "Advanced Mammalian Gene Transfer: High Titre Retroviral Vectors With Multiple Drug Selection Markers and a Complementary Helper-Free Packaging Cell Line," <i>Nucl. Acids Res.</i> , 18(12):3587-3595 (1990).	
C67	Mount, S.M., "A Catalogue of Splice Junction Sequences," <i>Nucl. Acids Res.</i> , 10(2):459-472 (1982).	
C68	Mustonen <i>et al.</i> , "Endothelial Receptor Tyrosine Kinases Involved in Angiogenesis," <i>J. Cell Biol.</i> , 129:895-898 (May, 1995).	
C69	Nelson and Sun, "The 50- and 58-kdalton Keratin Classes as Molecular Markers for Stratified Squamous Epithelia: Cell Culture Studies," <i>J. Cell Biol.</i> , 97:244-251 (July, 1983).	
C70	Neufeld <i>et al.</i> , "Vascular Endothelial Growth Factor and Its Receptors," <i>Prog. Growth Fact. Res.</i> , 5:89-97 (1994).	
C71	Oefner <i>et al.</i> , "Crystal Structure of Human Platelet-derived Growth Factor BB," <i>EMBO J.</i> , 11(11):3921-3926 (1992).	
C72	Oelrichs <i>et al.</i> , "NYK/FLK-1: A Putative Receptor Tyrosine Kinase Isolated from E10 Embryonic Neuroepithelium is Expressed in Endothelial Cells of the Developing Embryo," <i>Oncogene</i> , 8:11-18 (1993).	

EXAMINER <i>[Signature]</i>	DATE CONSIDERED <i>[Signature]</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)

U.S. Department of Commerce
Patent and Trademark Office

App. Docket No. 28967/32863	Serial No. 08/510,133
Applicant Alitalo and Joukov	
Filing Date 08/01/95	Group 1814

INFORMATION DISCLOSURE STATEMENT

(Use several sheets if necessary)



OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

C73	Olofsson <i>et al.</i> , "Vascular Endothelial Growth Factor B, A Novel Growth Factor for Endothelial Cells," <i>Proc. Nat'l Acad. Sci., USA</i> , 93:2576-2581 (March, 1996).
C74	Paavonen <i>et al.</i> , "Chromosomal Localization and Regulation of Human Vascular Endothelial Growth Factors B and C (VEGF-B and VEGF-C)," <i>IX International Vascular Biology Meeting</i> , Seattle, Washington, September 4-8, 1996, p. 76 (ABSTRACT 299).
C75	Paavonen <i>et al.</i> , "Novel Human Vascular Endothelial Growth Factor Genes VEGF-B and VEGF-C Localize to Chromosomes 11q13 and 4q34, Respectively," <i>Circulation</i> 93(6):1079-1082 (March 15, 1996).
C76	Park <i>et al.</i> , "Placenta Growth Factor, Potentiation of Vascular Endothelial Growth Factor Bioactivity In vitro and In vivo, and High Affinity Binding to Flt-1 but not to Flk-1/KDR," <i>J. Biol. Chem.</i> , 269(41):25646-25654 (October 14, 1994).
C77	Partanen <i>et al.</i> , "A Novel Endothelial Cell Surface Receptor Tyrosine Kinase with Extracellular Epidermal Growth Factor Homology Domains," <i>Mol. & Cell. Biol.</i> , 12(4):1698-1707 (April, 1992).
C78	Partanen <i>et al.</i> , "Putative Tyrosine Kinases Expressed in K-562 Human Leukemia Cells," <i>Proc. Nat'l Acad. Sci., USA</i> , 87:8913-8917 (November, 1990).
C79	Paulsson <i>et al.</i> , "The Balbiani Ring 3 Gene in <i>Chironomus tentans</i> has a Diverged Repetitive Structure Split by Many Introns," <i>J. Mol. Biol.</i> , 211:331-349 (1990).
C80	Pear <i>et al.</i> , "Production of High-titer Helper-free Retroviruses by Transient Transfection," <i>Proc. Nat'l Acad. Sci., USA</i> , 90:8392-8396 (September, 1993).
C81	Pertovaara <i>et al.</i> , "Vascular Endothelial Growth Factor Is Induced in Response to Transforming Growth Factor- β in Fibroblastic and Epithelial Cells," <i>J. Biol. Chem.</i> , 269(9):6271-6274 (March 4, 1994).
C82	Peters <i>et al.</i> , "Vascular Endothelial Growth Factor Receptor Expression during Embryogenesis and Tissue Repair Suggests a Role in Endothelial Differentiation and Blood Vessel Growth," <i>Proc. Nat'l Acad. Sci., USA</i> , 90:8915-8918 (October, 1993).

EXAMINER	DATE CONSIDERED
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 08/01/95	Group 1814

16
MAIL ROOM
FEB 13 1995
OTHER DOCUMENTS (including Author, Title, Date, Pertinent Pages, etc.)

C83	Pötgens <i>et al.</i> , "Covalent Dimerization of Vascular Permeability Factor/Vascular Endothelial Growth Factor Is Essential for Its Biological Activity," <i>J. Biol. Chem.</i> , 269(52):32879-32885 (December 30, 1994).
C84	Puri <i>et al.</i> , "The Receptor Tyrosine Kinase TIE is Required for Integrity and Survival of Vascular Endothelial Cells," <i>EMBO J.</i> , 14:5884-5891 (1995).
C85	Quinn <i>et al.</i> , "Fetal Liver Kinase 1 is a Receptor for Vascular Endothelial Growth Factor and is Selectively Expressed in Vascular Endothelium," <i>Proc. Nat'l Acad. Sci., USA</i> , 90:7533-7537 (August, 1993).
C86	Risau <i>et al.</i> , "Platelet-Derived Growth Factor is Angiogenic <i>In Vivo</i> ," <i>Growth Factors</i> , 7:261-266 (1992).
C87	Risau, W., "Differentiation of Endothelium," <i>FASEB J.</i> , 9:926-933 (1995).
C88	Sabin, F.R., "The Lymphatic System in Human Embryos, With A Consideration of the Morphology of the System as a Whole," <i>Am. J. Anat.</i> , 9(1):43-91 (1909).
C89	Sambrook <i>et al.</i> , <i>Molecular Cloning: a Laboratory Manual</i> , Second Edition, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory (1989), pp. 2.60-2.79, 4.21-4.32, 7.3-7.36, and 9.47-9.51.
C90	Sato <i>et al.</i> , "Distinct Roles of the Receptor Tyrosine Kinases Tie-1 and Tie-2 in Blood Vessel Formation," <i>Nature</i> , 376:70-74 (July 6, 1995).
C91	Schneider <i>et al.</i> , "A One-step Purification of Membrane Proteins Using a High Efficiency Immunomatrix," <i>J. Biol. Chem.</i> , 257(18):10766-10769 (September 25, 1982).
C92	Seetharam <i>et al.</i> , "A Unique Signal Transduction from FLT Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor VEGF," <i>Oncogene</i> , 10:135-147 (1995).
C93	Senger <i>et al.</i> , "Tumor Cells Secrete a Vascular Permeability Factor That Promotes Accumulation of Ascites Fluid," <i>Science</i> , 219:983-985 (February 25, 1983).
C94	Shalaby <i>et al.</i> , "Failure of Blood-Island Formation and Vasculogenesis in Flk-1-deficient Mice," <i>Nature</i> , 376:62-66 (July 6, 1995).

EXAMINER

DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	App. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 08/01/95	Group 1814

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
C95	Shibuya <i>et al.</i> , "Nucleotide Sequence and Expression of a Novel Human Receptor-Type Tyrosine Kinase Gene (<i>flt</i>) Closely Related to the <i>fms</i> Family," <i>Oncogene</i> , 5:519-524 (1990).	
C96	Shibuya, M., "Role of VEGF-FLT Receptor System in Normal and Tumor Angiogenesis," <i>Adv. Cancer Res.</i> , 67:281-316 (1995).	
C97	Shweiki <i>et al.</i> , "Patterns of Expression of Vascular Endothelial Growth Factor (VEGF) and VEGF Receptors in Mice Suggest a Role in Hormonally Regulated Angiogenesis," <i>J. Clin. Invest.</i> , 91:2235-2243 (May, 1993).	
C98	Southern and Berg, "Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter," <i>J. Mol. Appl. Genet.</i> , 1:327-341 (1982).	
C99	Terman <i>et al.</i> , "Identification of New Endothelial Cell Growth Factor Receptor Tyrosine Kinase," <i>Oncogene</i> , 6:1677-1683 (1991).	
C100	Terman <i>et al.</i> , "Identification of the KDR Tyrosine Kinase as a Receptor for Vascular Endothelial Cell Growth Factor," <i>Biochem. Biophys. Res. Commun.</i> , 187:1579-1586 (September 30, 1992).	
C101	Terman <i>et al.</i> , "VEGF Receptor Subtypes KDR and FLT1 Show Different Sensitivities to Heparin and Placenta Growth Factor," <i>Growth Factors</i> , 11(3):187-195 (1994).	
C102	Tischer <i>et al.</i> , "The Human Gene for Vascular Endothelial Growth Factor. Multiple Protein Forms are Encoded Through Alternative Exon Splicing," <i>J. Biol. Chem.</i> , 266(18):11947-11954 (June 25, 1991).	
C103	Vassar <i>et al.</i> , "Tissue-specific and Differentiation -specific Expression of a Human K14 Keratin Gene in Transgenic Mice," <i>Proc. Nat'l Acad. Sci., USA</i> , 86:1563-1567 (March, 1989).	
C104	Vassar <i>et al.</i> , "Transgenic Mice Provide New Insights Into the Role of TGF- α During Epidermal Development and Differentiation," <i>Genes & Dev.</i> , 5:714-727 (1991).	

EXAMINER	DATE CONSIDERED
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

INFORMATION DISCLOSURE STATEMENT

(Use several sheets if necessary)

OTHER DOCUMENTS - (Including Author, Title, Date, Pertinent Pages, etc.)

[illegible]

EXAMINER

DATE CONSIDERED _____

⁴EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name: I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed on August 1, 1995, as Application Serial No. 08/510,133. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment attached hereto. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Priority Claimed
☐ ☒

950624	Finland	13 February 1995	Yes	No
(Application Serial Number)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

	(Day/Month/Year Filed)
(Application Serial Number)	

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

08/340,011	14 November 1994	Pending
(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)
(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shulman (19,412)	Trevor B. Joike (25,542)	Richard A. Schnurr (30,890)	James J. Napoli (32,361)
Donald J. Bost (19,490)	Timothy J. Vezeau (26,348)	Anthony Nimmo (30,920)	Richard M. La Barge (32,254)
Owen J. Murray (22,111)	Carl E. Moore, Jr. (26,487)	Christine A. Dudzik (31,245)	Jeffrey W. Smith (33,455)
Allen H. Gerstein (22,218)	Richard H. Anderson (26,526)	Kevin D. Hogg (31,839)	Douglass C. Hochstetler (33,710)
Nate F. Scarpelli (22,320)	Patrick D. Ertel (26,877)	Jeffrey S. Sharp (31,879)	Cynthia L. Schaller (34,245)
Edward M. O'Toole (22,477)	James P. Zeller (28,491)	Donald J. Pochopien (32,167)	Robert M. Gerstein (34,824)
Michael F. Borun (25,447)	William E. McCracken (30,195)	Martin J. Hirsch (32,237)	David A. Gass (38,153)

Send correspondence to: David A. Gass

FIRM NAME	PHONE NO.	STREET	CITY & STATE	ZIP CODE
Marshall, O'Toole, Gerstein, Murray & Borun	312-474-6300	6300 Sears Tower 233 South Wacker Drive	Chicago, Illinois	60636-6402

Full Name of First or Sole Inventor Kari Alitalo	Citizenship Finland
Residence Address - Street Nyyrikintie 4A	Post Office Address - Street Same
City (Zip) 02100 Espoo	City (Zip) Same
State or Country FINLAND	State or Country Same
Date 08	Signature 08

See second page for additional inventor

See reverse for relevant rules & statutes

APPLICABLE RULES AND STATUTE

37 CFR 1.56. DUTY OF DISCLOSURE - INFORMATION MATERIAL TO PATENTABILITY (Applicable Portion)

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentability defines, to make sure that any material information contained therein is disclosed to the Office.

Information relating to the following factual situations enumerated in 35 USC 102 and 103 may be considered material under 37 CFR 1.56(a).

35 U.S.C. 102. CONDITIONS FOR PATENTABILITY: NOVELTY AND LOSS OF RIGHT TO PATENT

A person shall be entitled to a patent unless --

- (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent, or
- (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States, or
- (c) he has abandoned the invention, or
- (d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months before the filing of the application in the United States, or
- (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraph (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or
- (f) he did not himself invent the subject matter sought to be patented, or
- (g) before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other.

35 U.S.C. 103. CONDITIONS FOR PATENTABILITY; NON-OBVIOUS SUBJECT MATTER

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negative by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

35 U.S.C. 112. SPECIFICATION (Applicable Portion)

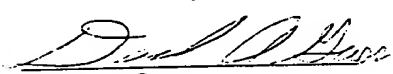
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Second Joint Inventor, if any Vladimir Joukov	Citizenship Russia
Residence Address - Street Topeliuksenkatu 32G8	Post Office Address - Street Same
City (Zip) 00290 Helsinki	City (Zip) Same
State or Country FINLAND	State or Country Same
Date <input checked="" type="checkbox"/> Aug. 6, 1996	Signature <input checked="" type="checkbox"/> V. Joukov



Handwritten: 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100
PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)	I hereby certify that this paper is
Serial No: 08/510,133)	being deposited with the United
Filed: August 1, 1995)	States Postal Service with sufficient
Title: RECEPTOR LIGAND)	postage as first class mail in an
Group Art Unit: 1814)	envelope addressed to: Assistant
Examiner: Lathrop, B.)	Commissioner for Patents,
)	Washington, D.C., 20231 on this
)	date:
)	
)	Date: February 10, 1997
)	
)	
)	David A. Gass
)	Registration No. 38,153
)	Attorney for Applicants

REQUEST FOR AMENDMENT OF DRAWING

Assistant Commissioner for Patents
Washington, D.C. 20231

Attn: Official Draftsman

Dear Sir:

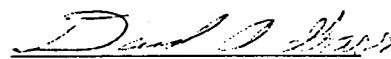
The Applicants respectfully request entry of an amendment to Figure 10 of the above-identified application. Three copies of Amended Figure 10 are attached hereto. Also attached is a copy of Figure 10 as filed, marked in red ink to show the amendments thereto.

Amended Figure 10 differs from Figure 10 as originally filed only in that two "N" residues have been underlined in amended Figure 10. The two underlined "N" residues conform to art-recognized glycosylation sequences (i.e.,

Asn-X-Ser or Asn-X-Thr). Support for this amendment is found at page 26, lines 21-24, in the specification. Entry of this amendment is requested to improve conformity between the drawing and the specification as filed.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN



David A. Gass
Registration No. 38,153
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

Chicago, Illinois
February 10, 1997

	1					50
PDGF-A	.MRTWACLLL	LGCGYLAHAL	AEAEIIPREL	IERLARSQIH	SIRDLQRLLE	
PDGF-B	MNRCAW.LFL	SLCCYLRLVS	AEGDPIPEEL	YEMLS DHSIR	SFDDLQRL LH	
PlGFMP	VMRLFPCLQ	LLAGLAL...	
VEGFMNFLLSWVH	WSLALLLYLH	
FLT4-L	MTVLYPEYWK	MYKCQLRKG	
	51					100
PDGF-A	IDSVGAEDAL	ETSLRAHGS	AINHVPEKRP	VPIRRKRSI.EEAIP	
PDGF-B	GDP.GEEDGA	ELDLNMTRSH	SGGELES...	.LARGRRSLG	SLTIAEPAMI	
PlGF	PAVPPQOWAL	SA.....	GNGSSEVEVV	P.FQEVWG...R	
VEGF	HAKWSQAAPM	AE.....	GGQNHHEVV	K.FMDVYQ...R	
FLT4-L	WQHNREQANL	NSRTEETIKF	AAAHYNTAIL	KSIDNEW...K	
	101					150
PDGF-A	AVCKTRTVIY	EIPRSQVDPT	SANFLIWEPC	VEVKRCIGCC	NTSSVKQPS	
PDGF-B	AECKTRTEVF	EISRRLLDRT	NANFLVWEPC	VEVKRCIGCC	NNRNVCORPT	
PlGF	SYRALERLV	DVVSEY..PS	EVEHMFSPSC	VSLLRIGCC	GDENLHVPV	
VEGF	SYCHPIETLV	DIFOEY..PD	EIEYIFKESC	VPLMRGCGCC	NDEGLECVPT	
FLT4-L	TCMPREVC	DVGKEF..GV	ATNTFFKEPC	VSRYRCGGCC	NSEGLCMNT	
	151					200
PDGF-A	RVHHRSVKVA	KVEYVRKKPK	LKEVQVRLEE	HLPCA....	AT.....	
PDGF-B	QVQLRPVQVR	KIEIVRKKPI	FKKATVTLED	HLACKC....	ETVAAARPVT	
PlGF	ETANVTMQLL	KIRSG..DRP	.SYVELTFSQ	HVRLECRPLR	EKMKPERC..	
VEGF	EESNITMQIM	RIKPH..OGQ	.HIGEMSFLQ	HNKCECRPKK	DRARQENP..	
FLT4-L	STSYLSKTLF	EITVPLSQGP	.KPVTFISFAN	HTSFCRMSKL	DVYRQVHSII	
	201					250
PDGF-A	..SNLNPDDR	EEETDVR...	
PDGF-B	RSPGGSQEQR	AKTPQTRVTI	RTVRVRPPK	GKHKFKHHTH	DKTALKETLG	
PlGF	GDAVPRR...	
VEGF	CGPCSERRKH	LFVQDPQTCK	CSCKNTDSRC	KAROLELNER	
FLT4-L	RRSLPATLPQ	CQAANKTCPT	NYMWNHICR	CLAQEDFMFS	SDAGDDSTDG	
	251					300
PDGF-A	
PDGF-B	A.....	
PlGF	
VEGF	TCRC DKPRR.	
FLT4-L	FHDICGPNKE	LDEETCQCVC	RAGLRPASCG	PHKELDRNSC	QCVCKNKLFP	
	301					350
PDGF-A	
PDGF-B	
PlGF	
VEGF	
FLT4-L	SQCGANREFD	ENTCQCVCCKR	TCFRNQPLNP	GKCACECTES	PQKCLLKGGK	
	351					395
PDGF-A	
PDGF-B	
PlGF	
VEGF	
FLT4-L	FHHQTCSCYR	RPCTNRQKAC	EPGFSYSEEV	CRCVPSYWKR	PQMS	

FIGURE 10



PATENT
ATTORNEY DOCKET NO. 28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):) Title: RECEPTOR LIGAND
Alitalo et al.)
Serial No: 08/510,133) Group Art Unit: 1814
Filed: August 1, 1995) Examiner: Lathrop, B.

RECEIVED

FEB 27 1997

Group Art Unit

**AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME**

*Assistant Commissioner for Patents
Washington, D.C. 20231*

Sir:

Transmitted herewith for filing in the above-identified application are the following:

1. Amendment and Reply Pursuant to 37 C.F.R. §§1.111 and 1.115;
2. Information Disclosure Statement, including Form PTO-1449 and copies of documents B1-B5 and C14- C110;
3. Check for \$390.00 in payment of fee for Two Months Extension of Time;
4. Check for \$226.00 in payment of fee for extra claims;
5. Check for \$230.00 in payment of fee or consideration of IDS;
6. Copy of Inventors' Declaration; and
7. Request for Amendment of Drawing, including amended Figure 10 and copy of Figure 10 as filed, marked to show amendments.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on **February 10, 1997**, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231

David A. Gass

1. **Small Entity Status**

- ☐ Verified statement(s) claiming small entity status is(are) attached.
- ☐ Small entity status has been established and is still effective.
- ☒ Has not been established.

2. **Extension of Time**

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month		\$110.00		\$55.00
Two Months	x	\$390.00		\$195.00
Three Months		\$930.00		\$465.00
Four Months		\$1,470.00		\$735.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$390.00

- ☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$ _____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$

Extension Fee Due With This Request \$

3. Fee for Claims

The fee for additional claims ((37 CFR 1.16(b)-(d)) has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	23	MINUS	20	= 3	X11 =		X22 =	\$ 66.00
INDEP.	5	MINUS	3	= 2	X40 =		X80 =	\$160.00
11 First Presentation of Multiple Dependent Claim					+ 130 =		+ 260 =	
TOTAL ADDITIONAL FEE							OR	\$226.00

4. Method of Payment of Fees

- ☒ Attached is a check in the amount of: \$226.00
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

5. Deposit Account and Refund Authorization

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

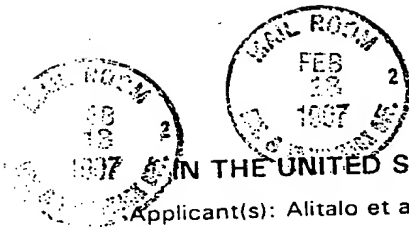
Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 

David A. Gass
Reg. No: 38,153

February 10, 1997



PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.

Serial No: 08/510,133

Filed: August 1, 1995


Title: RECEPTOR LIGAND

Group Art Unit: 1814

Examiner: Lathrop, B.

I hereby certify that this paper is
being deposited with the United
States Postal Service with sufficient
postage as first class mail in an
envelope addressed to: Assistant
Commissioner for Patents,
Washington, D.C., 20231 on this
date:

Date: February 10, 1997


David A. Gass
Registration No. 38,153
Attorney for Applicants

AMENDMENT AND REPLY PURSUANT TO 37 C.F.R. §§ 1.111 AND 1.115

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In an Office action mailed September 10, 1996, the Patent Office
allowed claims 2 and 12, but rejected claims 1, 8-10, and 13-19 variously under
35 U.S.C. §§ 102(a), 102(b), and 112, first and second paragraphs. The
Applicants respectfully request reconsideration in light of the following
amendments and remarks.

U.S. PATENT OFFICE
FEB 23 1997

AMENDMENTS

In the specification:

At page 3, line 30, after "ingrowth stage" please insert a period -- .

At page 6, line 19, please delete "election" and substitute therefor -- electron --.

At pages 12, lines 9, 10, and 19, please delete "ug" and in each instance substitute therefor -- μ g --.

At page 13, line 22, please delete "S68203" and substitute therefor -- X68203 --.

At page 17, line 14, please delete "recombinant-produced" and substitute therefor -- recombinantly-produced --.

At page 19, line 2, after "lanes 8 and 9" please insert -- in Figure 6

At page 19, line 27, please delete "ug" (both instances), and in each instance substitute therefor -- μ g --.

At page 20, line 11, and page 21, line 17, please delete "ug" and in each instance substitute therefor -- μ g --.

At page 21, line 16, please delete "design" and substitute therefor -- designed --.

At page 24, line 11, please delete "5xSSPE" and substitute therefor -- 5x SSPE --.

At page 24, line 12, please delete "1xSSC" and substitute therefor -- 1x SSC --.

At page 25, line 22, please delete "used". In the same line, please delete "and to" and substitute therefor -- and used to --.

At page 26, line 2, please delete "ul" and substitute therefor -- μ l --

At page 26, line 24, please delete "marked" and substitute therefor -- underlined --.

At page 26, line 28, please delete "BRF3" and substitute therefor -- BRP3 --.

At page 27, line 25, please delete "COOH-terminal" and substitute therefor -- carboxy-terminal --.

At page 27, line 28, please delete "asd" and substitute therefor -- as --.

At page 28, line 29, please delete "42C" and substitute therefor -- 42°C --.

At page 29, line 3, please delete "52C" and substitute therefor -- 52°C --.

At page 28, line 4, please delete "70C" and substitute therefor -- 70°C --.

In the drawing:

Please delete Figure 10 and substitute therefor amended Figure 10 filed herewith.

In the claims:

Please cancel claims 10 and 18; amend claims 1, 8, 9, 14, and 17; and add new claims 20-25 as shown below:

1. (Twice amended) A purified and isolated polypeptide [which specifically binds] capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase.

8. (Twice amended) A polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding [fragment of the purified and isolated polypeptide according to claim 2 which is capable of specifically binding to an Flt4 receptor tyrosine kinase].

9. (Twice amended) [The fragment] A polypeptide according to claim 8 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

14. (Amended) A purified and isolated polypeptide [according to claim 13] which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

17. (Amended) A purified and isolated polypeptide according to claim 1, said polypeptide being purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix

comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

-- 20. A polypeptide according to claim 8 which is capable of binding the extracellular domain of Flt4 receptor tyrosine kinase with high affinity and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

21. A polypeptide according to claim 8 further comprising a detectable label.

22. A pharmaceutical composition comprising a polypeptide according to claim 8 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

23. A polypeptide according to claim 14 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

24. A polypeptide according to claim 14 comprising a portion of SEQ ID NO: 33 effective to permit binding to Flt4 receptor tyrosine kinase and stimulation of Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

25. A pharmaceutical composition comprising a polypeptide according to claim 14 in a pharmaceutically-acceptable diluent, adjuvant, or carrier. --

REMARKS

I. History of claims and explanation of amendments.

The application as filed contained twelve claims. In a preliminary amendment (Paper No. 10) dated August 12, 1996, claims 1-4, 6, 8-9, and 12 were amended, and claims 13-19 were added to the application. In the present amendment, the Applicants cancel claims 10 and 18; amend claims 1, 8, 9, 14, and 17; and add new claims 20-25. Thus, claims 1-9, 11-17, and 19-25 are pending. Claims 3-7 and 11 have been withdrawn from consideration as being drawn to a non-elected invention.

All of the amendments herein find support in the application as originally filed. Most of the amendments to the specification correct obvious typographical and grammatical errors and the like, as requested by the Patent Office in paragraph 10 of the Office action. Support for the amendment at page 13, line 22, is found in the specification at page 13, line 18. Support for the amendment at page 19, line 2, is found in the specification at page 18, lines 16-24, for example.

Amended Figure 10 differs from Figure 10 as originally filed only in that two "N" residues have been underlined in amended Figure 10. The two underlined "N" residues conform to art-recognized N-linked glycosylation sequences (i.e., Asn-X-Ser or Asn-X-Thr). Support for this amendment is found at page 26, lines 21-24 in the specification. A separate letter requesting amendment of the drawing has been filed herewith.

The amendments to claim 1 find support in Example 5 (e.g., at p. 17, lines 13-15); at p. 4, line 6; and elsewhere throughout the specification.

The amendments to claim 8 and 14, which formerly depended from claims 2 and 1, find support in claims 2 and 1 as originally filed, as well as elsewhere throughout the specification.

The amendments to claim 17 find support in Example 5 (e.g., at p. 17, lines 13-15) and elsewhere throughout the specification.

The Applicants do not intend by these or any other amendments to abandon the subject matter of any claim as originally filed or later amended, and

reserve the right to claim such subject matter in other applications, such as continuations, continuations-in-part, and divisional applications.

II. The Patent Office's basis for maintaining the restriction requirement is inconsistent with the patent statutes.

In paragraphs 1 and 2 of the Office action, the Patent Office maintained the Restriction Requirement first imposed in the Office Action mailed May 29, 1996, citing as support various provisions of the M.P.E.P., including §802.01. The Applicants respectfully submit that, to the extent that the statutory provisions of 35 U.S.C. §121 ("independent and distinct") are inconsistent with the M.P.E.P. provisions cited by the Examiner ("may properly be divided if . . . 'distinct' inventions, even though dependent"), it is the statutory provisions which are controlling, because statutory provisions have the force of law. "The Manual does not have the force of law or the force of the Patent Rules of Practice in Title 37 Code of Federal Regulations." (See Forward to M.P.E.P., 6th Ed., Rev. 2, July, 1996.) Because the asserted basis for maintaining the restriction requirement is contrary to the plain language of the governing patent statute, reconsideration and withdrawal of the restriction requirement is respectfully requested.

III. The requirement that non-elected claims be canceled is premature.

In paragraph 3 of the Office action, the Patent Office required cancellation of non-elected claims or other appropriate action:

This application contains claims 3, 4, 6 (each amended), 5, 7, and 11 drawn to inventions non-elected with traverse in Paper No. 9. A complete response to the final rejection must include cancellation of non-elected claims or other appropriate action (37 C.F.R. §1.144; M.P.E.P. §821.01).

(Office action at p. 3.)

The Applicants respectfully submit that the outstanding Office action is the first Office action on the merits and is not a "final rejection." Only the restriction requirement has been made final. Accordingly, the requirement that the

withdrawn claims be canceled, as part of a "complete response to the final rejection," is premature.

- IV. Several claims in the application are entitled to priority based on U.S. Patent Application Serial No. 08/340,011, when one considers the entirety of the '011 application, including the preliminary amendment thereto.

In paragraph 6 of the Office action the Patent Office asserted that "no claims are afforded priority to application Serial No. 08/340011" (hereinafter "the '011 application"). (Office action at p. 4.) The Applicants respectfully traverse, because several claims in the application are entitled to priority based on the '011 application, when one considers the entirety of the '011 application, including the preliminary amendment thereto.

- A. The '011 priority application contains numerous teachings related to an Flt4 ligand that the Patent Office failed to consider in its priority determination.

The '011 application was a Rule 62 continuation-in-part of an earlier application, and was filed with a preliminary amendment containing a disclosure of an Flt4 ligand. (See preliminary amendment to the '011 application dated November 14, 1994.) The Patent Office's stated rationale for its determination that the '011 application affords no priority to the present application cites only pages 7-8 of the '011 application, suggesting that the more pertinent preliminary amendment was never considered:

Examples 12-17 in the '011 application all were introduced in the preliminary amendment portion thereof, and all are highly pertinent to the claim of priority in the present application. For example, Example 12 in the '011 application teaches that a conditioned media from the PC-3 prostatic adenocarcinoma cell line (ATCC CRL 1435) is capable stimulating Flt4 autophosphorylation in NIH3t3 cells expressing Flt4. Example 12 further teaches that a "flow-through fraction" of concentrated conditioned medium (i.e., the fraction containing proteins of less than 10,000 molecular weight) was not responsible for stimulating the Flt4 phosphorylation, and that pretreatment of the conditioned medium with Flt4 extracellular domain (coupled to CNBr-

activated Sepharose) is capable of removing the component responsible for stimulating Flt4. (Preliminary amendment to '011 application at p. 11.)

Based on the experimental results reported in Example 12, the '011 application states, "These data prove that PC-3 cells produce soluble ligand for FLT4. The above experiments prove that the ligand binds to the recombinant FLT4 EC domain. Thus, that ligand can be purified using the recombinant FLT4 EC domain in affinity chromatography. The purified protein can be electrophoresed in SDS-PAGE, blotted onto polyvinylidene difluoride (PVDF) membranes and its amino terminal sequence can be determined by methods standard in the art." (Preliminary amendment to '011 application at p. 11.) Thereafter, Example 12 contains teachings as to the determination of peptide sequences of the purified ligand and identification and cloning of a cDNA encoding the ligand.

Example 15 in the '011 application is directed to "Purification and sequencing of the Flt4 ligand." (Preliminary amendment to '011 application at p. 15.) Example 15 teaches that the PC-3 conditioned medium is concentrated and loaded onto a column of immobilized Flt4 extracellular domain. One embodiment taught in Example 15 is an affinity matrix comprising the Flt4 extracellular domain cross-linked to CNBr-activated Sepharose, i.e., the same affinity matrix employed in Example 5 in the present application. Example 15 in the '011 application teaches that chromatographic fractions are tested for the ability to stimulate tyrosine phosphorylation of Flt4, as was done in Example 5 of the present application. Thus, Example 15 in the '011 priority document teaches an affinity purification procedure for purifying a Flt4 ligand of the invention.

Example 15 in the '011 application further directs, "The purified biologically active ligand protein is microsequenced and the degenerate oligonucleotides are made based on the amino acid sequence obtained." (Preliminary amendment to '011 application at p. 15.) Example 16 of the '011 application is directed to constructing a cDNA library from PC-3 cells, to be screened for a cDNA encoding the Flt4 ligand. (Compare Example 6 in the present application.) Example 17 in the '011 application provides procedures

for screening the cDNA library using, e.g., oligonucleotide probes generated based upon the peptide sequences of purified Flt4 ligand.

Significantly, the '011 application also contained claims to an Flt4 ligand and to methods of using an Flt4 ligand. (See preliminary amendment to '011 application at pp. 18-19.) Uses for an Flt4 ligand that are taught in the '011 application include use for the detection of Flt4 (increased Flt4 expression being observable in metastatic lymph nodes and lymphangiomias); use for regulating the growth and functions of certain endothelial cells, especially lymphatic endothelia; and use for assaying for inhibitors. (See, e.g., Preliminary Amendment to '011 application at pp. 6-7 and 19.) Thus, when one considers the entirety of the '011 application, including its preliminary amendment, one finds abundant §112, first paragraph, support for a purified Flt4 ligand as claimed in the present application.

.B. Several claims in the present application are properly afforded priority to the '011 application.

Referring to the present application, claim 1 is directed to "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase." As explained in detail in subpart A, above, the '011 application teaches how to make and to use such a polypeptide. Accordingly, claim 1 is afforded priority by the '011 application.

Claim 13 depends from claim 1 and contains the limitation that the polypeptide have "an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions." The approximate 23 kD molecular weight is an *inherent property* of the Flt4 ligand that the '011 application teaches one how to purify from the PC-3 conditioned medium. As such, the inclusion of this property in the present application and in claim 13 does not deprive claim 13 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*, 33 U.S.P.Q.2d 1274, 1276, 44 F.3d 988 (Fed. Cir. 1995) ("A claim in a CIP application is entitled to the filing date of the parent application when the claimed invention is described in the parent specification in a manner that satisfies, inter alia, the description requirement of

35 U.S.C. §112. . . . [T]he later explicit description of an inherent property does not deprive the product of the benefit of the filing date of the earlier application."); see also *Application of Davies*, 177 U.S.P.Q. 381, 385, 475 F.2d 667, 671-72 (C.C.P.A. 1973) ("[W]e see no impediment to the present applicants' refiling their application and incorporating a discussion of the allegedly unobvious properties [of the invention] while retaining the effective date of the application involved here through §120.")

Claim 16 depends from claim 13 and recites that "amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13." The recited amino acid sequence is an *inherent property* of the Flt4 ligand that the '011 application teaches one how to purify from PC-3 conditioned medium.¹ As such, the inclusion of this property in the present application and in claim 16 does not deprive claim 16 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*, and *Application of Davies, supra*.

Claim 17 depends from claim 1 and requires that the polypeptide be purifiable from PC-3 conditioned media (PC-3 cell line ATCC CRL No. 1435) using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase. As explained in detail in subpart A, above, the '011 application teaches in detail the procedure recited in claim 17 for purification of such a polypeptide. Accordingly, claim 17 is afforded priority by the '011 application.

Claim 19 depends from claim 1 and requires a detectable label. Detectably labeled Flt4 ligands were specifically contemplated as an aspect of the '011 application. (See e.g., Preliminary Amendment to '011 application at pp. 18-19 (claims 33-35).) Accordingly, claim 19 is afforded priority by the '011 application.

Independent claim 14 of the present application recites, "A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine

¹ Moreover, the '011 application teaches that the amino terminal amino acid sequence should be determined. (See Preliminary Amendment to '011 application at p. 15 (Example 15).)

kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase. As explained in part A, above, the '011 application teaches how to make and use such a polypeptide. Accordingly, claim 14 is afforded priority by the '011 application. Claim 23, which depends from claim 14 and contains a molecular weight limitation ("approximately 23 kD"), is afforded priority to the '011 application for the reasons described above for claim 13.

As the foregoing discussion of the '011 preliminary amendment indicates, several claims in the present application, including at least claims 1, 13, 14, 16, 17, and 19, are entitled to priority from the '011 application. The Applicants respectfully request appropriate correction of the Patent Office's priority determination in the next action on the merits in the present application.

V. A copy of the Inventors' supplemental declaration has been filed herewith.

In paragraph 7 of the Office action, the Examiner indicated that the supplemental inventor's declaration filed with the Preliminary Amendment dated August 12, 1996, was misplaced or omitted during transmittal. A copy of the supplemental inventor's declaration is filed herewith to complete the Patent Office's file.

VI. The Applicants will file formal drawings upon receipt of a notice of allowability.

In paragraph 8 of the Office action the Patent Office suggests that, "Pursuant to a change in Office policy effective 25 April 1996, *formal drawings will be required at the time allowable subject matter is first indicated.*" Notwithstanding the foregoing, and the fact that claims 2 and 12 in the application have been allowed, the Applicants submit that formal drawings are not required until issuance of a Notice of Allowability, i.e., until the application is allowed. See 37 C.F.R. §1.85 and M.P.E.P. §608.02(b). In a telephone interview between the Examiner and the undersigned attorney, which the Applicants acknowledge with thanks, the Examiner confirmed that formal drawings were not required in response to the outstanding Office action.

VII. The objection to the terminology at page 27, line 24, should be withdrawn.

In paragraph 9 of the Office action, the Examiner asserted that less than preferable terminology was employed at page 27, line 24, of the specification:

The disclosure is objected to because of the following informalities: 3' deletions of the Flt4 ligand are taught at p. 27, line 24, but deletions occurring at the end of a protein are preferably termed "C-Terminal" or "carboxy terminal" deletions. "3'" is preferred for describing nucleic acids only. Appropriate correction is required.

(Office action at p. 5.)

The applicants respectfully submit that page 27, lines 24-26, refer to "progressive 3' deletions in the 3' coding sequences of the Flt4 ligand precursor clone, resulting in COOH-terminal truncations of its protein product." Thus, the specification uses the phrase "3' deletions" to describe alteration of a nucleic acid encoding a protein, and use the phrase "COOH-terminal truncations" for describing deletions at the end of the protein encoded thereby. In the present amendment, the Applicants have deleted "COOH-terminal" and substituted therefor the "carboxy-terminal" language preferred by the Patent Office. Accordingly, the specification is in conformity with the preferred language noted by the Patent Office, and the objection thereto should be withdrawn.

VIII. The objection to claim 17 should be withdrawn because claim 17 provides further limitation to claim 1 from which it depends.

In paragraph 11 of the outstanding Office action, the Patent Office objected to claim 17, asserting that claim 17 failed to further limit claim 1 from which claim 17 depends:

Claim 17 is objected to under 37 C.F.R. §1.75(c) for not further limiting the subject matter of claim 1.

Specifically, the instant claim does not recite further structural limitations to the polypeptide of claim 1; the particular source of the polypeptide is *de minimus* [sic].

(Office action at p. 6.)

The Applicants respectfully traverse.

Claim 17 recites, "A purified and isolated polypeptide according to claim 1, *said polypeptide being purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase* . Thus, whereas claim 1 is directed to any purified and isolated polypeptide that is capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, claim 17 further requires that the polypeptide be purifiable from a conditioned media from a particular cell line. The Applicants respectfully submit that not every polypeptide that satisfies the requirements of claim 1 is also purifiable from conditioned media of the particular cell line recited in claim 17. Thus, the limitation as to source carries with it inherent structural limitations: the structure of the Flt4 ligand polypeptides that are isolatable from PC-3 medium. Since claim 17 further limits claim 1, the objection thereto should be withdrawn.

IX. The objection to claim 14 has been rendered moot.

In paragraph 12 of the Office action, the Examiner objected to claim 14 under 37 C.F.R. §1.175(c), asserting that the claim failed to limit the subject matter of claim 13. (Office action at p. 6.) Claim 14 has been amended herein to read as an independent claim. Accordingly, the Patent Office's objection is rendered moot, and should be withdrawn.

In its objection, the Patent Office also made assertions concerning the issue of enabling disclosure:

Claim 14 recites the functional limitation of stimulating Flt4 phosphorylation, which is a functional limitation not enabled by the teachings of the specification (M.P.E.P. §2173.05(g)). The specification teaches a polypeptide of 23 kD that stimulates Flt4 phosphorylation at Figure 5. The specification does not teach necessary or sufficient structures of the 23 kD protein that promote phosphorylation, nor could the skilled artisan have predicted what these structures would be from the state of the art at the time of the invention.

(Office action at p. 6.)

The Applicants respectfully traverse.

As recognized by the Patent Office, "The specification teaches a polypeptide of 23 kD that stimulates Flt4 phosphorylation." Thus, the Patent Office acknowledges that the specification contains a working example of a polypeptide meeting the "capable of stimulating Flt4 phosphorylation" limitation of claim 14.

Additionally, the specification teaches *in vitro* assays useful for screening polypeptides for their ability to stimulate Flt4 phosphorylation. (See, e.g., Example 4 (pp. 15-17) of the specification. The teaching of an Flt4 phosphorylation assay serves to precisely define the metes and bounds of claim 14, making the functional language perfectly acceptable. See M.P.E.P. §2173.05(g). As outlined in the remarks below (see Parts XI and XII) concerning the issue of enabling disclosure, the specification satisfies the enabling disclosure requirements of the patent statute by virtue of its teachings relating to Flt4 ligands, coupled with its teachings relating to screening assays involving Flt4 protein (or the extracellular domain thereof). Accordingly, the Applicants respectfully request withdrawal of the Patent Office's remarks relating to non-enablement.

X. The rejections of claims 1 and 8-10 under 35 U.S.C. §112, second paragraph, as being indefinite should be withdrawn.

In paragraphs 13-15 of the Office action, the Patent Office rejected claims 1 and 8-10, asserting that these claims were indefinite. Claims 9-10 and 13-19 also were rejected because of their dependence from claims 1 and/or 8. As set forth below, the foregoing amendments to the claims render all of these rejections moot.

A. The rejection of claims 1 and 8 should be withdrawn.

In paragraph 13, the Patent Office rejected claims 1 and 8, asserting that the term "specifically" therein rendered the claims indefinite:

The term "specifically" in claims 1 and 8 is a relative term which renders the claim indefinite. The term "specifically" is not defined by the claim, the specification

does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

The art can define high affinity and non-specific binding to receptors; however, the instant inventions encompasses numerous polypeptides which would be expected to have affinities for the receptor anywhere between high affinity to non-specific binding. Intermediate affinities are not definable without a standard of affinity for "specific" binding, and hence it is impossible to determine whether such compounds would be included within the bounds of the claims.

(Office action at pp. 6-7.)

Contrary to the Patent Office's assertions, one skilled in the art understands from the specification that "specific" binding is an indication of high affinity binding. For example, Example 5 teaches that an approximately 23 kD polypeptide is isolatable from PC-3 conditioned medium via affinity chromatography, using the Flt4 extracellular domain in the affinity matrix. This polypeptide was found only in chromatographic fractions associated with Flt4 stimulating activity. All other components in the chromatographic fractions containing stimulatory activity were also distributed in the starting material and in small amounts in other washing and elution steps. "Similar results were obtained in three independent affinity purifications, indicating that the 23 kD polypeptide specifically binds to Flt4." (Specification at p. 19, lines 6-8.) Thus, the specification provides an indication that the ability to affinity purify is a measure of specific binding. The specification also provides the guidance that the isoforms of VEGF "do not show specific binding to Flt4." (Specification at p. 4, lines 24-25.)

The Patent Office's own rejection is evidence that "specific" binding is understood to be an indication of high affinity binding. For example, the Patent Office asserts, "The art can define high affinity and non-specific binding to receptors." (Office action at p. 7.) Here, the Patent Office acknowledges that the art can define "high affinity binding" to receptors and can define its antithesis, "non-specific binding to receptors." Implicit in the Patent Office's own reasoning is an understanding that "specific" binding is high affinity binding, which the art can define. Thus, the Office action itself

demonstrates that the indefiniteness objection to the phrase "specifically binds" is without merit.

Notwithstanding the foregoing, the Applicants have adopted the Patent Office's suggested language and have amended claim 1 herein to recite, "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase." The adoption of the "high affinity" language -- which was suggested by the Patent Office and which "the art can define" -- renders the rejection of claim 1 moot.

The amendments set forth herein also render the objection moot with respect to claim 8. Claim 8 has been amended to recite, "A polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding." Thus, claim 8 requires binding to Flt4 and requires that the polypeptide comprise a portion of SEQ ID NO: 33 effective to permit such binding. Because claim 8 no longer recites "specifically binds," the rejection based upon indefiniteness is rendered moot.

B. The rejection of claim 10 has been rendered moot.

In paragraph 14 of the Official action, the Patent Office rejected claim 10, asserting that the term "approximately" in claim 10 rendered the claim indefinite. (Office action at p. 7.) Claim 10 has been canceled herein without prejudice, rendering this rejection moot.

C. The rejection of claims 8-10 relating to the term "fragments" has been rendered moot.

In paragraph 15 of the Office action, the Patent Office rejected claims 8-10, asserting that the claims were indefinite:

Claims 8-10 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, fragments of a polypeptide comprising SEQ ID NO:33 are claimed. As such, the fragments must consist of *at least* the amino acids of SEQ ID NO:33. It is unclear whether this is the intended meaning, because the

fragments of claims 9 and 10, for example, appear to encompass only part of SEQ ID NO:33.

(Office action at p. 8.)

The Applicants respectfully submit that the definition of the term "fragment" asserted by the Patent Office is contrary to the understanding of the artisan of ordinary skill. However, claim 10 has been canceled without prejudice and claims 8 and 9 have been amended such that neither claim recites "fragment." Accordingly, the rejection for indefiniteness has been rendered moot.

D. Summary

All of the rejections under § 112, second paragraph have been rendered moot by the amendments herein. Accordingly, these rejections should be withdrawn.

XI. The rejection of claims 8 and 9 under § 112, first paragraph, should be withdrawn because the specification enables one to make the invention commensurate in scope with these claims.

In paragraph 17 of the Office action, the Patent Office rejected claims 8 and 9 under 35 U.S.C. § 112, first paragraph, asserting that the specification fails to enable one skilled in the art to make the invention commensurate in scope with these claims:

Claims 8 and 9 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for the scope of claim 10, does not reasonably enable the range of polypeptides encompassed in the instant claims. The specification does not enable any person skilled in the art to which it pertains, or with it is most nearly connected, to make the invention commensurate in scope with these claims.

(Office action at p. 10.)

The Applicants respectfully traverse.

Amended claim 8 recites, "A polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding." Claim 9, which depends from claim

8, requires that the polypeptide have an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

By providing the amino acid sequence set forth in SEQ ID NO: 33, the specification enables one skilled in the art to make essentially any polypeptide comprising a portion of SEQ ID NO: 33. For example, such polypeptides may be synthesized using automated peptide synthesizers or using recombinant techniques (e.g., using polynucleotides of the invention). Moreover, the specification enables binding assays to determine whether a polypeptide that has been synthesized is capable of binding to Flt4 receptor tyrosine kinase, and enables phosphorylation assays to determine whether such a polypeptide is capable of stimulating Flt4 autophosphorylation (see, e.g., Example 4). For these reasons alone, the specification enables one to make the invention of claims 8 and 9, and the rejection under § 112, first paragraph, must be withdrawn.

Moreover, the Patent Office's reasoning does not support the present rejection of the amended claims. The Patent Office's first basis for rejection is that "claim 8 encompasses fragments of all sizes from all locations of the protein of SEQ ID NO:33, and claim 9 encompasses all of these same fragments having an apparent molecular weight of 23 kD." However, claim 8 does not encompass fragments of all sizes and locations of the protein of SEQ ID NO: 33. Rather, claim 8 encompasses only polypeptides which comprise *a portion of SEQ ID NO: 33 effective to permit binding to an Flt4 receptor tyrosine kinase*. As the Patent Office acknowledges in its rejection, the skilled artisan would not predict that any fragment of the protein comprising SEQ ID NO:33 would bind the receptor. Thus, the scope of amended claim 8 (and claim 9 which depends therefrom) is narrower than the Patent Office has asserted.²

² By teaching that polypeptides of the invention are Flt4 ligands, and by teaching how to make and use Flt4 protein (and/or the extracellular fragment thereof), the specification enables one skilled in the art to perform the routine screening necessary to identify those portions of SEQ ID NO: 33 that are effective to permit Flt4 binding.

The Patent Office's second asserted basis for rejection is set forth below:

Second, the specification teaches at Example 5 a purified polypeptide having an apparent molecular weight of approximately 23 kD and an N-terminal sequence consisting of SEQ ID NO:13. The specification also teaches at Example 11 that a fragment having amino acids 1-180 of SEQ ID NO:33 *may* bind the receptor.

(Office action at p. 10.)

The foregoing quotation is a description of a working example and of guidance provided in the specification for identifying portions of SEQ ID NO: 33 effective to permit Flt4 binding. As such, the foregoing quotation supports, rather than negates, a conclusion of enablement. See *Ex parte Forman* (cited by the Patent Office),

The Patent Office's remaining rationales in support of its rejection relate to the alleged unpredictability of identifying effective portions of SEQ ID NO: 33:

Third, the skilled artisan could not predict which of the vast number of polypeptides encompassed by the claims would bind the Flt4 receptor, because the secondary structures required for interacting with the receptor were unknown and the secondary structures of any of the fragments of the claimed polypeptide were unknown. As Ferrara et al. teach at column 29, lines 29-32, even endothelial growth factors of the same size may have widely different structures and potencies. Thus, the skilled artisan would not predict that any fragment of the protein comprising SEQ ID NO:33 would bind the receptor solely because it's 23 kD in size. Fourth, a vast amount of experimentation would be required to make all the encompassed fragments and test their ability to bind the receptor. Thus, an undue amount of experimentation would be required to make and use the claimed invention.

(Office action at p. 10.)

However, these assertions ignore guidance provided in the specification for determining which portions of SEQ ID NO: 33 are required to permit binding to Flt4, and further ignore the nature of experimentation that one skilled in the art would conduct to identify such fragments.

The specification provides significant guidance for determining portions of SEQ ID NO: 33 that are effective to permit Flt4 binding. For example, although SEQ ID NO: 33 contains 350 amino acids, the specification provides guidance that the region critical for receptor activation is contained within its first approximately 180 amino acid residues.

By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin, et al., Growth Factors 8, 245-252, 1993) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues.

(Specification, pp. 27-28.)

Additionally, the specification outlines a protocol for defining that portion of SEQ ID NO: 33 which corresponds with the naturally-occurring Flt4 ligand. (See p. 27, lines 5-22.) Third, the specification provides guidance to (a) generate progressive deletion products of the Flt4 ligand cDNA; (b) express these modified cDNAs; and (c) assay the resulting truncated protein forms, e.g., by studying their ability to induce Flt4 autophosphorylation. These teachings serve to both provide guidance for predicting the portions of SEQ ID NO: 33 that are effective to permit Flt4 binding; and (2) reduce the amount of experimentation required to determine the minimum portion of SEQ ID NO: 33 that is critical for receptor binding.

Importantly, because the specification teaches *in vitro* screening assays (employing Flt4 or Flt4 extracellular domain), the experimentation required to practice the full scope of claims 8 and 9 is routine in nature. The fact that routine screening assays are what is required, and that such assays are taught in the specification, further supports a conclusion of enablement. See *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988) ("Enablement is not precluded by the necessity for some experimentation such as routine screening.

... The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.") The Patent Office's rationale that "a vast amount of experimentation would be required to make all the

encompassed fragments and test their ability to bind the receptor" ignores the fact that the required experimentation is rendered routine by the Flt4 *in vitro* screening assays of the specification.

The Patent Office's rationale that "a vast amount of experimentation would be required to make all the encompassed fragments and test their ability to bind the receptor" also ignores the scientific ability of one of ordinary skill in the art. Importantly, one of ordinary skill in the art would not conduct experimentation by haphazardly making all of the possible fragments of SEQ ID NO: 33 and testing their ability to bind the receptor. An artisan of ordinary skill understands that each fragment that is screened provides guidance as to that portion of SEQ ID NO: 33 that is effective for binding, and that portion which is not.³ An artisan of ordinary skill also understands techniques for accelerating a screening process,⁴ and techniques for screening multiple polypeptides *simultaneously*. Thus, the Patent Office's reasoning greatly overstates both the quantity and the nature of the experimentation required to practice the invention as claimed.

For all of these reasons, the specification enables one of ordinary skill in the art to practice the invention of claims 8 and 9, and the rejection under §112, first paragraph, should be withdrawn.

³ For example, a determination that a polypeptide comprising residues 34-180 of SEQ ID NO: 33 is effective to permit binding to Flt4 and that a polypeptide comprising residues 181-350 is ineffective to permit binding would provide significant guidance as to that portion of SEQ ID NO: 33 to further screen for effective fragments. Thus, the assertion that it would be necessary to screen "all" fragments of SEQ ID NO: 33 to practice the claimed invention relies upon the false assumption that individual screening assays will be performed without knowledge gained from prior screenings.

⁴ For example, it is within the skill of the art to synthesize spaced deletion mutants (e.g., residues 34-350, 34-330, 34-310, etc.) from SEQ ID NO: 33, rather than successive deletion mutants (34-350, 34-349, 34-348 . . .), to more rapidly identify effective portions for binding Flt4.

XII. The rejection of claims 1, 13-15, and 17-19 under §112, first paragraph, should be withdrawn because the specification enables one to make the invention commensurate in scope with these claims.

In paragraph 16 of the Office action, the Patent Office rejected claims 1, 13-15, and 17-19 under 35 U.S.C. §112, first paragraph, asserting that the specification fails to enable one skilled in the art to make the invention commensurate in scope with these claims:

Claims 1, 13-15 and dependent claims 17-19 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for the scope of claim 16, does not reasonably enable the range of polypeptides encompassed in the instant claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

(Office action at p. 8.)

The Applicants traverse in part and amend in part.

A number of the Patent Office's bases for rejecting claims 1, 13-15, and 17-19 relate to the amount of experimentation required to make and test polypeptides. As explained in detail in Part XI, above, the experimentation that is required is routine screening, and the specification enables *in vitro* screening assays which employ Flt4 protein or the extracellular domain thereof. In such circumstances, the Patent Office's reviewing court tolerates a considerable amount of experimentation under §112, first paragraph. See *In re Wands, supra*. Under the guidelines established in *In re Wands*, the conclusion that *undue* experimentation is required was improper.

Moreover, the Applicants have amended claims 1, 14, and 17 herein to overcome the Patent Office's rejections. In its rejection, the Patent Office asserts, "claim 1 encompasses all proteins that may interact with the Flt4 receptor." However, claim 1 is not directed to all proteins that may interact with the Flt4 receptor, but only to "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase." Thus, amended claim 1 is commensurate in scope with the specification, which teaches a high affinity ligand which binds the extracellular domain of Flt4.

Similarly, claim 14 has been amended to recite, "A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase. Thus, amended claim 14 requires more than mere "relatively weak" interaction with Flt4; amended claim 14 requires interaction that stimulates Flt4 receptor phosphorylation, as exemplified in the application.

Claim 17 has been amended to recite, "A purified and isolated polypeptide according to claim 1, said polypeptide being purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line . . . using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase." Amended claim 17 also is commensurate in scope with the teachings in the application. Even if, as the Patent Office asserts, many different growth factors were found in PC-3 conditioned medium, one would not expect the many different growth factors to bind Flt4 with sufficient affinity to permit affinity purification. Claim 17 is directed only to those polypeptides which are purifiable from such a medium using Flt4 affinity purification. The specification contains a working example of such an affinity purification. (See Example 5, pp. 17-19.)

The remaining rejected claims all depend from amended claims 1, 14, or 17. Thus, the amendments to claims 1, 14, and 17 are sufficient to overcome the Patent Office's rejections of all of claims 1, 13-15, and 17-19, and the rejections should be withdrawn.

XIII. The rejection under §102(a) based upon Pajusola *et al.* was improper, because the cited reference fails to disclose a purified polypeptide capable of binding with high affinity to Flt4.

In paragraph 19 of the Office action, the Patent Office rejected claim 1 under §102(a), asserting that the claim is anticipated by Pajusola *et al.*:

Claim 1 is rejected under 37 U.S.C. § 102(a) as being anticipated by Pajusola *et al.*

Pajusola *et al.* teach at p. 3550, column 1, a purified polypeptide, colony stimulating factor-1 (CSF-1), which binds a CSF-1 receptor/Flt4 fusion protein. An inherent

property of the fusion protein is the Flt4 receptor tyrosine kinase. Pajusola et al. thus anticipate claim 1.

(Office action at p. 11.)

Claim 1 has been amended herein to recite, "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase."

Pajusola et al., *Oncogene*, 9:3545-3555 (1994) discloses that colony stimulating factor-1 (CSF-1) specifically activated a fusion protein comprising the ligand binding extracellular domain of the colony stimulating factor-1 receptor (CSF-1R) fused to the transmembrane and cytoplasmic domains of an Flt4 receptor. (See, e.g., p. 3549, col. 2.) The fusion protein was expressed in specified cell lines. The transgenic cells expressing the fusion protein were stimulated using CSF-1.

One skilled in the art understands that CSF-1 binds to the CSF-1 receptor, and more particularly to the extracellular domain of the CSF-1 receptor. The receptor chimera in Pajusola et al. comprised the extracellular domain of the CSF-1 receptor and the transmembrane and intracellular domains of the Flt4 receptor. Thus, it is apparent to one skilled in the art that when CSF-1 is shown to bind this CSF-1R/Flt4 chimera in transfected cells, the binding occurs between the CSF-1 and the CSF-1 receptor extracellular domain portion of the chimera. Thus, Pajusola et al. discloses a purified polypeptide which binds to a chimeric polypeptide comprising the extracellular domain of CSF-1R. Pajusola et al. fails to disclose or suggest a purified polypeptide that binds to Flt4. Accordingly, the rejection of claim 1, which requires a purified and isolated polypeptide capable of binding with high affinity to Flt4 receptor tyrosine kinase, must be withdrawn.

Moreover, amended claim 1 requires a purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase. The chimeric receptor described in Pajusola et al. does not include the extracellular domain of Flt4. For this additional reason, the anticipation rejection of claim 1, based upon Pajusola et al., must be withdrawn.

Finally, as stated above, claim 1 is entitled to a priority date of November 14, 1994, the filing date of the priority document. Accordingly, Pajusola *et al.*, stated by the Patent Office to have been published in December, 1994, does not constitute prior art.

For all of these reasons, the rejection under §102(a) must be withdrawn.

XIV. The rejection under §102(b) has been rendered moot.

In paragraph 20 of the Office action, the Patent Office rejected claim 18 under §102(b), asserting that the claim is anticipated by Sitaras *et al.*:

Claim 18 is rejected under 35 U.S.C. §102(b) as being anticipated by Sitaras *et al.*

Sitaras *et al.*, the whole document, teach a conditioned medium, thus anticipating the claimed invention. Because this conditioned medium is from PC-3 prostatic adenocarcinoma cells, it has the inherent property of comprising the polypeptide of claim 1.

(Office action at p. 11.)

The Applicants have canceled claim 18 herein, rendering this rejection moot.

XV. Applicants comments concerning the Examiner's statement of reasons for the indication of allowable subject matter.

In paragraph 22 of the Office action, the Examiner stated certain reasons for the indication of allowable subject matter in the present application. Included in the statement of reasons were characterizations of a Human Genome Sciences (HGS) PCT publication that does not constitute prior art:

Human Genome Sciences, Inc. published the sequence of a Flt4 receptor ligand with greater than 99 percent identity to the instant ligand, but the date of publication does not antecede the filing date of the instant application. The ligand of Human Genome Sciences, Inc. was purified using expressed sequence tags without identification as encoding a vascular endothelial growth factor; therefore, one of ordinary skill in the art at the time of the invention would not have been motivated to use these art-known sequences to arrive at the instant invention.

(Office action at p. 12.)

The Applicants respectfully submit that the foregoing characterization of the HGS publication (PCT publication WO95/24473) includes conclusions made *after reading the present application*, and that the HGS publication discloses less than the Examiner has indicated.

The Examiner stated that "Human Genome Sciences, Inc. published the sequence of a Flt4 receptor ligand with greater than 99 percent identity to the instant ligand." This characterization overstates the teachings in the HGS publication. Importantly, the HGS publication fails to disclose or suggest that any polypeptide or other molecule is *an Flt4 receptor ligand*. It is the **present application** that discloses that a polypeptide is a ligand for Flt4. The Examiner has concluded, based upon the similarity of an HGS sequence to a sequence disclosed by the present applicants, that the HGS sequence is that of an Flt4 ligand. However, the Examiner's familiarity with the present application was required for this conclusion. It is the present application, and not the HGS publication, which identifies an Flt4 receptor tyrosine kinase ligand.⁵

The Examiner also stated, "The ligand of Human Genome Sciences, Inc. was purified using expressed sequence tags" Apparently, the Examiner intended to state that the nucleotide sequence taught in the HGS publication was obtained using expressed sequence tags The HGS publication does not disclose "purification" of a "ligand." At best, Example 2 in the HGS publication purports to disclose in vitro transcription and translation of three polynucleotides and analysis of the reaction products on an SDS-PAGE gel. The HGS publication states that translated products with estimated molecular weights of "38-40 dk" and "36-38 kd" were observed on the gel.

⁵ As discussed in detail in preceding sections, the guidance provided in the present application that an *Flt4 ligand* has been identified has important legal ramifications under §112, first paragraph. For example, one would not be motivated to perform *in vitro* screening assays on fragments or variants of a protein, using Flt4 (or an extracellular fragment thereof), if one is unaware that the protein itself is an Flt4 ligand.

The failure of the HGS application to identify a receptor through which the putative "VEGF2 polypeptide" mediates any putative biological activity is significant, and clarification for the record is respectfully requested.


XVI. Summary

For the foregoing reasons, the Applicants respectfully request reconsideration, withdrawal of all claim rejections and objections to the specification and claims, and allowance of claims 1-9, 11-17, and 19-25.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Dated: February 10, 1997


David A. Gass
Registration No. 38,153
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300



9012

1801

PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:) I hereby certify that this paper
Alitalo et al.) is being deposited with the
Serial No.: 08/510,133) United States Postal Service as
Filed: August 1, 1995) first class mail, postage
For: RECEPTOR LIGAND) prepaid, in an envelope
Group Art Unit: 1814) addressed to: Assistant
Examiner: Lathrop, B.) Commissioner for Patents
Washington, D.C. 20231, on this
date:
Date: March 27, 1997
David A. Gass
David A. Gass
Registration No. 38,153
Attorney for Applicant(s)

STATEMENT CLAIMING SMALL ENTITY STATUS

Assistant Commissioner for Patents
Washington, D.C. 20231

Attn: Accounting Division
Office of Finance

Sir:

The Applicants hereby claim small entity status in
the above-identified matter for purposes of paying fees,
pursuant to 37 C.F.R. 1.27, based on the following documents
transmitted herewith:

- 1) Verified Statement (Declaration) Claiming
Small Entity Status (37 CFR 1.9(f) and
1.27(c)) -- Small Business Concern; and
- 2) Verified Statement (Declaration) Claiming Small
Entity Status (37 CFR 1.9(f) and 1.27(d)) --
Nonprofit Organization.

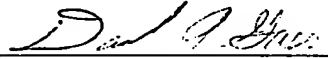
The Verified Statements are signed by Heikki Lampi, on behalf
of Helsinki University Licensing, Ltd., and Edward A.
McDermott, on behalf of Ludwig Institute for Cancer Research,

the co-owners of the patent application and of the invention described therein.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

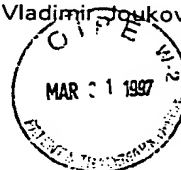
Dated: *March 27, 1997*


David A. Gass
Registration No. 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, IL 60606-6402
Telephone: (312) 474-6300

PATENT

Attorney's Docket No: 28967/32863

Applicant or Patentee: Kari Alitalo and Vladimir Joukov
Serial or Patent No: 08/510,133
Filed or Issued: August 1, 1995
For: Receptor Ligand



VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(d)) -- NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: Ludwig Institute for Cancer Research

ADDRESS OF ORGANIZATION: 1345 Avenue of the Americas
New York, NY 10105

TYPE OF ORGANIZATION

- ☐ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
- ☒ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3))
- ☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)
- ☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501 (a) and 501 (c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA
- ☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled RECEPTOR LIGAND, by inventor(s) Kari Alitalo and Vladimir Joukov

described in

- ☐ The specification filed herewith.
- ☒ Application Serial No. 08/510,133, filed August 1, 1995.
- ☐ Patent No. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization regarding the above-identified invention. If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights in the invention is listed below and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

FULL NAME: Helsinki University Licensing, Ltd.
ADDRESS: Viikinkaari 8 A, FIN-00710 Helsinki, Finland
☐ INDIVIDUAL ☒ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME: _____
ADDRESS: _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that

such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Edward A McDermott

TITLE IN ORGANIZATION: President

ADDRESS OF PERSON SIGNING: 1345 Avenue of the Americas, New York
NY 10105

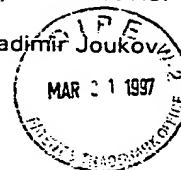
SIGNATURE: *Edward A. McDermott*

Date: March 2, 1997

PATENT

Attorney's Docket No: 28967/32863

Applicant or Patentee: Kari Alitalo and Vladimir Joukov
Serial or Patent No: 08/510,133
Filed or Issued: August 1, 1995
For: Receptor Ligand



VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(c)) -- SMALL BUSINESS CONCERN

I hereby declare that I am

- ☐ The owner of the small business concern identified below:
- ☒ An official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: Helsinki University Licensing, Ltd.

ADDRESS OF BUSINESS: Viikinkaari 8 A, FIN-00710 Helsinki, Finland

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, a concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to, and remain with, the small business concern identified above with regard to the invention, entitled Receptor Ligand, by inventor(s) Kari Alitalo and Vladimir Jovkov,

described in

- ☐ The specification filed herewith.
- ☒ Application Serial No. 08/510,133, filed August 1, 1995.
- ☐ Patent No. _____, issued _____.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).

NAME: Ludwig Institute for Cancer Research
ADDRESS: 1345 Avenue of the Americas, New York, NY 10105
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☒ NONPROFIT ORGANIZATION

NAME: _____
ADDRESS: _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Heikki Lampi

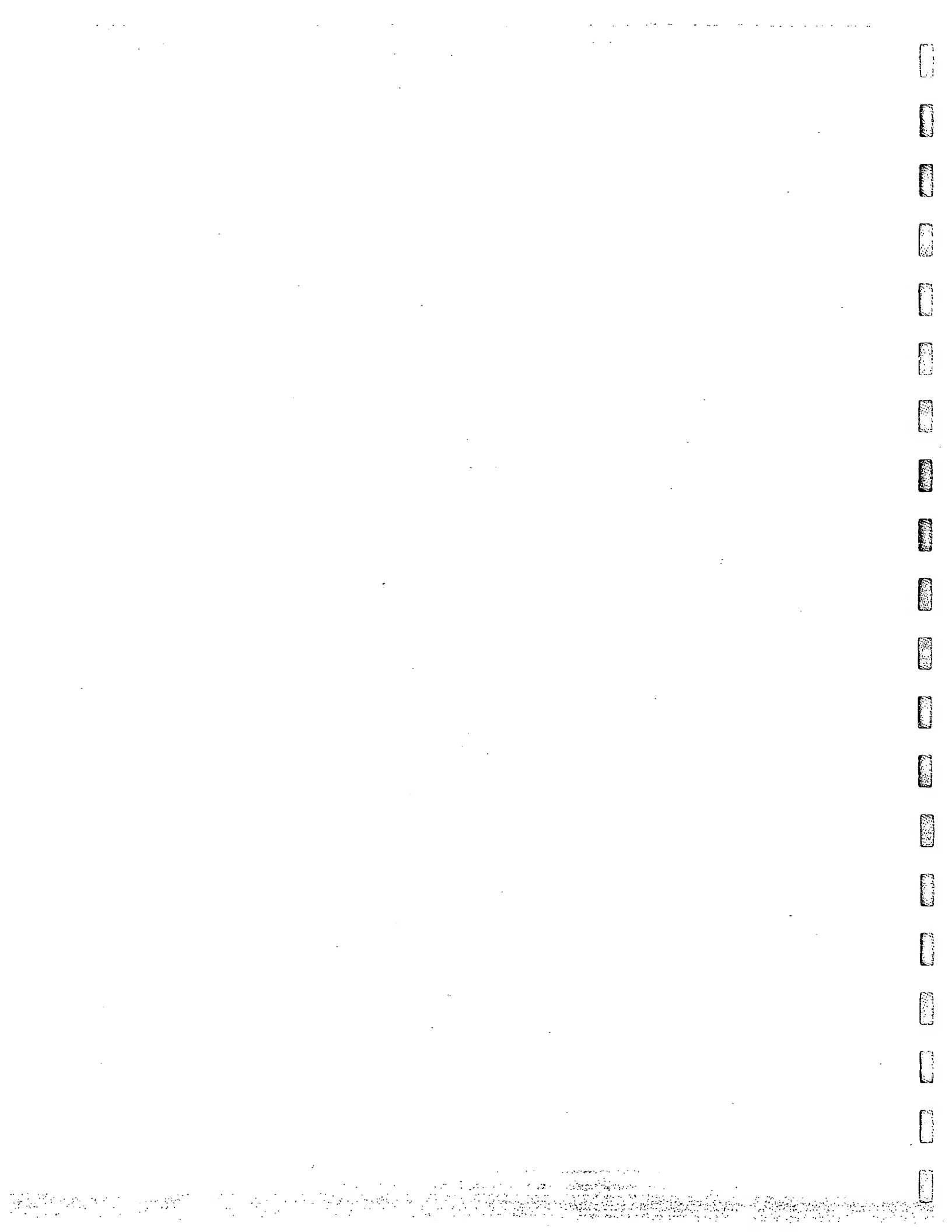
TITLE OF PERSON OTHER THAN OWNER: President

ADDRESS OF PERSON SIGNING: Viikinkaari 8 A, FIN-00710 Helsinki, Finland

SIGNATURE: _____

Date

22. Feb. 1997





PATENT APPLICATION
28967/32863

GROUP 1800

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitalo, Kari)	I hereby certify that this paper and
)	the documents referred to as enclosed
and Joukov, Vladimir)	herewith are being deposited with the
)	United States Postal Service as First
Serial No.: 08/510,133)	Class Mail, postage prepaid, in an
)	envelope addressed to: Assistant
Filed: August 1, 1995)	Commissioner for Patents,
)	Washington, DC 20231, on this date:
)	
For: "Receptor Ligand")	Date: <u>April 10, 1997</u>
)	<u>David A. Gass</u>
Group Art Unit: 1814)	David A. Gass
)	Reg. No.: 38,153
Examiner: B.K. Lathrop, Ph.D.)	Attorney for Applicants

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed documents, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of each itemized document is enclosed herewith. All of the documents were identified in an International Search Report (ISR, copy enclosed herewith) in a related PCT patent application. Documents identified in the ISR that are not itemized on the attached Form PTO-1449 have already been made of record by the Patent Office or the applicants.

This Information Disclosure Statement is not intended to be an admission that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

Pursuant to 37 C.F.R. §1.97(e)(1), the Applicants certify that each document itemized on the attached form PTO-1449 was cited in a communication (an ISR) from a foreign patent office (the European Patent Office) in a counterpart foreign (PCT) application, not more than three months prior to the filing of this statement. Accordingly, pursuant to 37 C.F.R. §1.97(c)(2), the information disclosed herein should be considered by the Patent Office without payment of any fee.

However, the Patent Office is hereby authorized to charge any fees due in connection with this paper to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: Apr 11, 1997

By: David A. Gass
David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

FILE COPY

#18 attached
SHEET 1 of 1

Form PTO-1449 (Rev. 10-1997)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 08/01/95	Group 1814



U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate

FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No
CA	B6	WO 95/33772	12/14/95	PCT			

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

CA	C111	Hillier et al., "The WashU-Merch EST Project," EMBL Database entry HS991157, accession no. H07991, July 2, 1995.

EXAMINER C. Saoud	DATE CONSIDERED 9/27/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	



SHEET 1 of 1

Form 9-O-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo and Joukov	
		Filing Date 08/01/95	Group 1814

19

U.S. PATENT DOCUMENTS							
*Examiner Initials		Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate

FOREIGN PATENT DOCUMENTS								
*Examiner Initials		Document Number	Publication Date	Country	Class	Subclass	Translation	
							Yes	No
	B6	WO 95/33772	12/14/95	PCT				

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
	C111	Hillier <i>et al.</i> , "The WashU-Merch EST Project," EMBL Database entry HS991157, accession no. H07991, July 2, 1995.

EXAMINER	DATE CONSIDERED
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

PATENT COOPERATION TREATY PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

RECEIVED
GROUP 1800

Applicant's or agent's file reference 28999	FOR FURTHER ACTION		see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/FI 96/00427	International filing date (day/month/year) 01/08/1996	(Earliest) Priority Date (day/month/year) 01/08/1995	
Applicant HELSINKI UNIVERSITY LICENSING LTD OY et al.			

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.
☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☐ Certain claims were found unsearchable (see Box I).
2. ☐ Unity of invention is lacking (see Box II).
3. ☐ The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing

☐ filed with the international application.
☐ furnished by the applicant separately from the international application,
 ☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

☐ Transcribed by this Authority
4. With regard to the title, ☒ the text is approved as submitted by the applicant.
 ☐ the text has been established by this Authority to read as follows:
5. With regard to the abstract, ☒ the text is approved as submitted by the applicant.
 ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.
6. The figure of the drawings to be published with the abstract is:
 Figure No. ☐ as suggested by the applicant. ☒ None of the figures.
 ☐ because the applicant failed to suggest a figure.
 ☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FI 96/00427

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/52 C07K19/00 C07K16/24 A61K38/19
G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ONCOGENE, vol. 10, 1995, pages 973-984, XP002022269 J.-P. BORG ET AL.: "Biochemical characterization of two isoforms of FLT4, a VEGF receptor-related tyrosine kinase" see the whole document, in particular the abstract and materials and methods sections. --- -/-	1-3,5, 18, 29-31,34

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

29 January 1997

Date of mailing of the international search report

0 5. 03. 97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Yeats, S

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FI 96/00427

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ONCOGENE, vol. 9, 1994, pages 3545-3555, XP002022270 K. PAJUSOLA ET AL.: "Signalling properties of FLT4, a proteolytically processed receptor tyrosine kinase related to two VEGF receptors" cited in the application see the whole document, in particular page 3553. ---	1-3,5, 18, 29-31,34
X	ONCOGENE, vol. 8, 1993, pages 2931-2937, XP002022271 K. PAJUSOLA ET AL.: "Two human FLT4 receptor tyrosine kinase isoforms with distinct carboxy terminal tails are produced by alternative processing of primary transcripts" cited in the application see the whole document, in particular page 2936. ---	1-3,5, 18, 29-31,34
X	EMBL Database entry HS991157, accession no. H07991, 2 July 1995; HILLIER L. ET AL. "The WashU-Merck EST Project" XP002022299 see the sequence. ---	37,38
P,X	WO,A,95 24473 (HUMAN GENOME SCIENCES, INC.) 14 September 1995 see the whole document, especially Figure 1 and the claims. ---	1-44
P,X	WO,A,95 33772 (K. ALITALO ET AL.) 14 December 1995 see Examples 8 and 9 and the claims. ---	1-3,5, 18,22, 29-31,34
P,X	EMBO J., vol. 15, 1996, pages 290-298, XP002022272 V. JOUKOV ET AL.: "A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGF-2) receptor tyrosine kinase" cited in the application see the whole document. -----	1-44

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/FI 96/00427

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9524473	14-09-95	AU-A- 7394194 EP-A- 0751992 ZA-A- 9403464	25-09-95 08-01-97 20-11-95
WO-A-9533772	14-12-95	AU-A- 2738895	04-01-96



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
03/310,133	08/01/95	ALITALO	K 28113/32863

18M2/0411
MARSHALL O'TOOLE GERSTEIN MURRAY
AND BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

EXAMINER

LATHROP, B

ART UNIT PAPER NUMBER

1801

DATE MAILED:

04/11/97

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

- ☒ Responsive to communication(s) filed on 4/13/97 (A-116)
- ☒ This action is FINAL.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or ~~thirty days~~, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

- ☒ Claim(s) 1-9, 11-17, 19-25 is/are pending in the application.
- ☐ Claim(s) 10, 26, 27 is/are withdrawn from consideration.
- ☒ Claim(s) 1-2 is/are allowed.
- ☒ Claim(s) 1, 8, 9, 13-15, 19-25 is/are rejected.
- ☒ Claim(s) 10, 17 is/are objected to.
- ☐ Other _____ are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some ☐ None of the CERTIFIED copies of the priority documents have been received.
- ☐ received in Application No. (Series Code/Serial Number) _____.
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
- *Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☐ Notice of Reference Cited, PTO-892
- ☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 13
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Notice of Informal Patent Application, PTO-152

- SEE OFFICE ACTION ON THE FOLLOWING PAGES -

Serial Number: 08/510133
Art Unit: 1801

-2-

DETAILED ACTION

Election/Restriction and Disposition of Claims

1. Applicants remarks concerning the finality of the requirement for restriction are noted.
- 5 Applicants argue that 35 USC 121 should be interpreted as requiring the inventions to be both "independent" and "distinct" to be properly restricted. The restriction requirement is final as set forth in Paper No. 11, mailed 9/10/96. Applicants are respectfully referred to the court's decision in *In re Lee*, 199 USPQ 108, 109 (ComrPats 1978).
- 10 2. Requirement of cancellation of non-elected claims is premature as noted by applicant and is withdrawn. Claims 1-9, 11-17, and 19-25 are pending, with claims 3-7 and 11 withdrawn from consideration as to a non-elected invention.

Priority

- 15 3. In support of their position that some claims may receive the benefit of priority under 35 USC 120, applicants point out that the examiner missed support for a Flt4 ligand in a preliminary amendment in application Serial No. 08/340011 ('011). The preliminary amendment in '011 describes conditioned medium from PC-3 cell cultures that comprises a soluble ligand for the Flt4 receptor (p. 11, last paragraph). '011 does not describe the ligand, but rather describes the
20 induction of receptor phosphorylation in response to a ligand and states that ligands may be purified from the conditioned medium (p. 6, penultimate paragraph). The examiner relies on the

Serial Number: 08/510133
Art Unit: 1801

-3-

decision in *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (CA FC 1993) as applied to the claimed ligand. The description in '011 of a conditioned medium containing a ligand or ligands that activate the Flt4 receptor and a potential method for isolating these ligands does not satisfy the written description requirement of 35 USC 112, because '011 does not describe the ligand itself,
5 nor does it demonstrate that the disclosed method would actually produce the claimed ligand. '011 therefore does not demonstrate that the inventor had possession of claimed ligand at the time it was filed. No claims are afforded priority to application Serial No. 08/340011 for the reasons set forth.

10 4. The examiner notes the mention of Finnish application Serial No. 950624 in the Declaration filed 2/27/97 without the claim of priority to this application under 35 USC 119.

Drawings

5. The corrected or substitute drawings were received on 2/13/97. These drawings are
15 acceptable.

6. Since allowable subject matter has been indicated, applicant is encouraged to submit formal drawings in response to this Office action. The early submission of formal drawings will permit the Office to review the drawings for acceptability and to resolve any informalities
20 remaining therein before the application is passed to issue. This will avoid possible delays in the

Serial Number: 08/510133
Art Unit: 1801

-4-

issue process. Applicants correctly note that formal drawings are not required at this stage in prosecution.

Withdrawn Objections and Rejections

5 7. The objections to claims 14 and 17 are withdrawn in view of applicants' arguments and
amendments. The remarks regarding the enabling disclosure are withdrawn from the
consideration of whether the claims should be objected to under 37 CFR 1.75 as not pertinent to
this Rule. Any remarks about the conformity of the claims to the enablement requirement will be
made as appropriate in the context of 35 USC 112, first paragraph, as set forth below. The
10 rejection of claims 1 and 8 and dependent claims 9 and 13-19 under 35 USC 112, second
paragraph, is withdrawn in view of applicants' arguments. The rejections of claim 10 under 35
USC 112, second paragraph, are rendered moot by the cancellation of claim 10. The rejection of
claims 8 and 9 under 35 USC 112, second paragraph, is withdrawn in view of applicants'
amendment. The rejection of claim 18 under 35 USC 112, first paragraph, is rendered moot by
15 the cancellation of the claim. The rejection of claim 1 under 35 USC 102(a) as being anticipated
by Pajusola et al. is withdrawn in view of applicants' amendment. The rejection of claim 18 under
35 USC 102(b) as being anticipated by Sitaras et al. is rendered moot by the cancellation of claim
18.

Claim Rejections - 35 USC § 101

20 8. 35 U.S.C. 101 reads as follows:

Serial Number: 08/510133
Art Unit: 1801

-5-

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

9. Claim 8 and dependent claims 9 and 20-22 are rejected under 35 U.S.C. 101 because the
5 claimed invention is directed to non-statutory subject matter. Claim 8 as amended no longer
recites a purified and isolated polypeptide and hence reads on a product of nature, which is non-
statutory subject matter.

Claim Rejections - 35 USC § 112

- 10 10. Claims 8, 9, and 20-22 are rejected under 35 U.S.C. 112, first paragraph, because the
specification, while being enabling for a portion of SEQ ID NO:33 capable of binding to an Flt4
receptor tyrosine kinase comprising about the amino terminal 180 amino acids, does not
reasonably provide enablement commensurate with the scope of the claims. The specification
does not enable any person skilled in the art to which it pertains, or with which it is most nearly
15 connected, to make the invention commensurate in scope with these claims.

Making the invention requires a portion of the protein of SEQ ID NO:33 effective to
permit binding to the Flt4 receptor. Claim 9 recites that this fragment must have an apparent
molecular weight of 23 kD determined by SDS-PAGE under reducing conditions. Claim 20
further requires that the fragment bind with high affinity and stimulate Flt4 receptor
20 phosphorylation. The specification guides the selection of Flt4 ligands comprising a portion of
SEQ ID NO:33 capable of binding the Flt4 receptor and stimulating its tyrosine kinase activity at
Example 11. The specification teaches that a 23 kD polypeptide from the protein of SEQ ID

Serial Number: 08/510133
Art Unit: 1801

-6-

NO:33 that is produced by 293-EBNA cells has biological activity, but that the protein of SEQ ID NO:33 is predicted to be about 35.7 kD in molecular weight. The specification does not teach which amino acids are present in the 23 kD fragment, nor does it teach whether post-translational modifications such as glycosylation may have changed the apparent molecular weight. The specification predicts without direct evidence that the first 180 amino acids of SEQ ID NO:33 will be sufficient for biological activity (p. 28, lines 1-3). Beyond this guidance, the specification provides no guidance to select fragments of the protein of SEQ ID NO:33 that are able to bind the Flt4 receptor. Without additional structural information on the ligand, the skilled artisan cannot predict which additional fragments of the protein of SEQ ID NO:33 might bind the receptor. Where the art is unpredictable, as in the case of physiological activity, more guidance is required. *In re Fisher*, 166 USPQ 18 (CCPA 1970). The vast amount of experimentation required to test all the encompassed fragments is one factor to be considered in the overall determination of whether the experimentation required to make the invention is undue. For the reasons set forth above, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

11. Applicant's arguments filed 2/13/97, Paper No. 15, have been fully considered but they are not persuasive. Applicants argue that the skilled artisan can make any fragment of the protein of SEQ ID NO:33 and that the determination of whether these fragments can bind the receptor requires routine experimentation. The examiner agrees that while fragments could be made, undue experimentation would be required to make those fragments *with the claimed biological*

Serial Number: 08/510133
Art Unit: 1801

-7-

activity of binding the Flt4 receptor given the guidance provided in the specification. Applicants argue that claim 8 encompasses only peptides capable of binding the receptor, and therefore is more limited in scope than the examiner contends. The examiner argues that the claim limitation of binding the receptor must be met by testing all fragments encompassed by the claims, which in this case are *not* limited in any way. Applicants argue that the guidance of the specification directs the artisan to try the amino terminal 130 amino acids, but they do not *claim* fragments comprising the first 180 amino acids of SEQ ID NO:33. Without this claim limitation, should the skilled artisan be guided by the fact that the only biologically active fragment shown is 23 kD in apparent molecular weight with an unknown degree of post-translational modification, or should they be guided by the supposed biological criticality of the amino terminal 180 residues? Applicants assert that remarks made by the examiner support the argument that there is enabled subject matter. The examiner agrees entirely but notes that the instant claims are not limited in scope to what the examiner indicated as enabled in his action mailed 9/10/96. Applicants argue that the assertion of unpredictability ignores the guidance in the specification, and that the alleged vast amount of experimentation required to make the experiment does not take the routine nature or the level of the skilled artisan into account. Unpredictability is generated by the uncertain result on biological activity when protein structure is altered, which may only be alleviated by providing some guidance as to which structures are necessary and sufficient for biological activity. The examiner has agreed that applicant has provided such guidance in the specification where the specification teaches the criticality of the first 180 residues for biological activity. While the quantity of experimentation is not *in itself* sufficient for determining undue experimentation, the

Serial Number: 08/510133
Art Unit: 1801

-8-

vast amount of experimentation required to test all the encompassed fragments is still correctly considered as a factor as noted by the examiner in the overall determination of whether the experimentation required to make the invention is undue. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

5

12. Claims 1, 13-15, 19, and 23-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the scope of claim 16 or claim 17, does not reasonably provide enablement commensurate with the scope of the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

10

The scope of claims 1, 13, 14, 19, 23, and 25 encompasses polypeptides from any source that bind with high affinity to the Flt4 receptor or stimulate its tyrosine kinase activity. Claims 13 and 23 require that these polypeptides have an apparent molecular weight of 23 kD on SDS-PAGE under reducing conditions. Making the invention requires testing all tissues from all known species, because neither the source nor the structure of the protein is recited in the instant claims. The teaching of the specification that the peptide is 23 kD provides no real guidance to make the invention commensurate with the scope of the claims, because many proteins are about this size, and the skilled artisan would be further burdened with determining the relative molecular weight of every protein from every tissue from every species known to meet the claim limitation.

20 There is no guidance provided by the specification to select those encompassed polypeptides that bind the Flt4 receptor with high affinity with the exception of those teachings which support the

subject matter indicated as enabled; i.e., the subject matter of claims 16 and 17. There is no guidance to predict *a priori* whether any protein would bind the receptor *without some information on the structure of the protein*, and this information was simply not available for all the proteins encompassed by the claims at the time of the invention. The scope of the required enablement varies inversely with the degree of predictability involved, and in applications directed to inventions in arts where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims. MPEP 2164.03 citing *In re Soll*, 97 F.2d 623, 38 USPQ 189 (CCPA 1938) and *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970).

10 The scope of claims 15 and 24 encompasses only those proteins comprising SEQ ID NO:13 or fragments of SEQ ID NO:33 capable of stimulating Flt4 receptor tyrosine kinase activity. As set forth above in regard to the activity of fragments of the protein of SEQ ID NO:33, the specification predicts without direct evidence that the first 180 amino acids of SEQ ID NO:33 will be sufficient for biological activity (p. 28, lines 1-3). Beyond this guidance, the
15 specification provides no guidance to select fragments of the protein of SEQ ID NO:33 that are able to bind the Flt4 receptor. Without additional structural information on the ligand, the skilled artisan cannot predict which additional fragments of the protein of SEQ ID NO:33 might bind the receptor. Where the art is unpredictable, as in the case of physiological activity, more guidance is required. *In re Fisher*, 166 USPQ 18 (CCPA 1970). The vast amount of experimentation
20 required to test all the encompassed fragments is one factor to be considered in the overall determination of whether the experimentation required to make the invention is undue.

Serial Number: 08/510133
Art Unit: 1801

-10-

For the reasons set forth, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

13. Applicant's arguments filed 2/13/97 have been fully considered but they are not persuasive.
- 5 Applicants argue that a considerable amount of experimentation is tolerated if that experimentation is routine in nature. While the quantity of experimentation is not *in itself* sufficient for determining undue experimentation, the vast amount of experimentation required to test all the encompassed fragments is still correctly considered as a factor as noted by the examiner in the overall determination of whether the experimentation required to make the
- 10 invention is undue. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988). Other remarks of applicants are made in regard to amendments to the claims and are fully considered in the rejection set forth above.

Allowable Subject Matter

- 15 14. Claims 2 and 12 are allowed. Applicants comments concerning WO 95/24473 are noted. By clarification, the examiner notes that this publication discloses a **polypeptide** with greater than 99% sequence identity to the instantly claimed protein of SEQ ID NO:33. Applicants correctly note that it was the polynucleotide disclosed in WO 95/24473 that was obtained from ESTs.

Serial Number: 08/510133
Art Unit: 1801

-11-

15. Claims 16 and 17 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Conclusion

16. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for response to this final action is set to expire **THREE**
10 MONTHS from the date of this action. In the event a first response is filed within **TWO**
MONTHS of the mailing date of this final action and the advisory action is not mailed until after
the end of the **THREE-MONTH** shortened statutory period, then the shortened statutory period
will expire on the date the advisory action is mailed, and any extension fee pursuant to 37
CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event will the
15 statutory period for response expire later than **SIX MONTHS** from the date of this final action.


17. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop, whose phone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

Serial Number: 08/510133
Art Unit: 1801

-12-

The examiner will attempt to respond to voice messages within 24 hours. Alternately, the examiner's supervisor, Vasu Jagannathan, can be reached at (703) 306-2777. The FAX number for Art Unit 1801 is (703) 305-7401.

5 An inquiry of a general nature relating to the status of this application should be directed to the Group 1800 receptionist whose telephone number is (703) 308-0196.


Brian K. Lathrop, Ph.D.

4/8/97

VASU S. JAGANNATHAN
PRIMARY EXAMINER
GROUP 1800



Initial Review
BOX AF

Response Under
37 C.F.R. 1.116 - Expedited
Procedure Examining
Group 1814

PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo *et al.*

Serial No: 08/510,133

Filed: August 1, 1995

Title: RECEPTOR LIGAND

Group Art Unit: 1814

Examiner: Lathrop, B.

) EXPRESS MAIL LABEL NO:

) EM099827086US

) Date of Deposit: June 11, 1997

) I hereby certify that this paper is being
) deposited with the United States Postal Service
) "EXPRESS MAIL POST OFFICE TO
) ADDRESSEE" service under 37 C.F.R. §1.10 on
) the date indicated above and is addressed to:
) Assistant Commissioner for Patents,
) Washington, D.C. 20231

) Mark Bonadonna

AMENDMENT AFTER FINAL ACTION

and

CONDITIONAL PETITION TO REVERSE OR WITHDRAWN ADVERSE PRIORITY
DETERMINATION PURSUANT TO
37 C.F.R. §1.181

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In an official action mailed April 11, 1997, the examiner finally rejected claims 1, 8, 9, 13-15, and 19-25 variously under 35 U.S.C. §§ 101 and 112, first paragraph. Claims 2 and 12 were allowed, and claims 16 and 17 were objected to as being dependent upon a rejected base claim, but were otherwise deemed allowable. The applicants respectfully request reconsideration in light of the following amendments and remarks.

AMENDMENTS

In the specification:

At page 24, line 30, after "Figure 9" please insert -- (SEQ ID NOs: 32 and 33) --.

In the claims:

Please cancel claims 3-7 and 11, without prejudice, amend claims 8, 16, 17, 21, 22, and 25, and add new claims 26-28, as shown below.

C¹ 8. (Three times amended) A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding.

C² 16. (Amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, [according to claim 13,] wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

17. (Twice amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, [according to claim 1,] said polypeptide being purifyable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

C³ 21. (Amended) A polypeptide according to claim [8] 17 further comprising a detectable label.

C³
ord.

22. (Amended) A pharmaceutical composition comprising a polypeptide according to claim ~~8~~ 17 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

C⁴

25. (Amended) A pharmaceutical composition comprising a polypeptide according to claim ~~14~~ 16 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

-- 26. A polypeptide according to claim 8 wherein said portion of SEQ ID NO: 33 effective to permit such binding is a continuous portion of SEQ ID NO: 33 within amino acids 1-180 of SEQ ID NO: 33.

C⁵

27. A polypeptide according to claim 8 wherein the amino terminus of said portion effective to permit such binding corresponds with position 34 of SEQ ID NO: 33.

28. A polypeptide according to claim 16 further comprising a detectable label. --

REMARKS

I. History of claims and explanation of amendments.

The application as filed contained twelve claims. In a preliminary amendment (Paper No. 10) dated August 12, 1996, claims 1-4, 6, 8-9, and 12 were amended, and claims 13-19 were added to the application. In an amendment dated February 10, 1997, the applicants canceled claims 10 and 18, amended claims 1, 8, 9, 14, and 17, and added new claims 20-25. In the present amendment, the applicants cancel claims 3-7 and 11, amend claims 8, 16, 17, 21, 22, and 25, and add new claims 26-28. Thus, upon entry of the foregoing amendments, claims 1-2, 8-9, 12-17, and 19-28 would be pending. A copy of the claims in their amended forms is appended hereto.

The nature of each claim amendment is discussed below in the remarks pertaining to each claim.

New claim 26 depends from claim 8 and further limits claim 8 by adopting a suggestion of the examiner with respect to subject matter that the specification enables. Support for the limitation "within amino acids 1-180 of SEQ ID NO: 33" is found in the specification at p. 28, lines 1-3. New claim 27 further limits claim 26 by reciting a specific amino terminal amino acid residue. The particular amino terminus that is recited in claim 27 corresponds to the amino terminus recited in claim 16. This amino terminus finds written support at p. 19, lines 17-19 of the specification.

II. Restriction Requirement

The applicants have canceled non-elected claims 3-7 and 11 without prejudice.

III. The Applicants respectfully request issuance of an advisory action wherein the Patent Office reverses as incorrect, or withdraws as inappropriate, its determination that no claims in the present application are afforded priority to U.S.S.N. 08/340,011.

In the outstanding official action, the examiner has asserted, for the first time, that no claims in the present application are entitled to priority based upon U.S.S.N. 08/340,011, filed November 14, 1994, *because of an asserted lack of written description* under 35 U.S.C. §112, first paragraph.¹ For the reasons set forth below, this determination is legally and factually incorrect. Moreover, the right of priority has no bearing on the patentability of any claim at

¹ In its first official action, the examiner made an initial determination that no claims were afforded priority by the '011 application, because of an *asserted absence of enabling disclosure*. However, that initial determination was made without any consideration of the preliminary amendment portion of U.S.S.N. 08/340,011 (a significant omission, since the '011 application is a Rule 62 continuation-in-part of an earlier application, and the preliminary amendment portion of the '011 application is highly pertinent to the priority issue). In the outstanding final action, the priority determination based on lack of enablement has properly been withdrawn. However, the examiner has, for the first time, raised a new objection to the priority claim, based upon an asserted lack of written description.

this time, and therefore, is an inappropriate subject for Patent Office determination.

- A. The applicants respectfully request entry into the record and consideration of the expert declaration of Dr. Carl-Henrik Heldin filed herewith.

The Patent Office's reviewing court has explicitly approved of the use of declarations which offer factual evidence to help resolve the issue of "written description" in a patent application, and has held that failure to accord appropriate weight to such declarations constitutes legal error. See *In re Alton*, 37 U.S.P.Q. 1578, 1583 (Fed. Cir. 1996). The applicants have filed herewith the expert declaration of Dr. Carl-Henrik Heldin (the "Heldin declaration") to offer a factual explanation as to why one of ordinary skill in the art would have understood the 1994 priority application to describe the invention presently being claimed. Since the examiner raised the written description issue for the first time in the outstanding final official action,² the applicants respectfully request entry of this declaration into the record and consideration thereof with respect to the issue of written description.

- B. The determination that no claims are entitled to priority is legally and factually incorrect.

The law is clear that original claims (i.e., claims contained in the patent application as filed) comply with the written description requirement of §112, because *original claims constitute their own description*. See *In re Koller*, 204 U.S.P.Q. 702, 706 (C.C.P.A. 1980). Moreover, later added claims of similar scope and wording are described by original claims. *Id.*

In the present case, the applicants' 1994 priority application (the '011 application) contained original claims to an Flt4 ligand. For example, original claim 31 recites, "A ligand which specifically binds to an FLT-4 receptor

² The written description issue was not necessitated solely by amendments made by the applicants in response to the first action on the merits, and therefore could have been raised by the Patent Office prior to the issuance of a final action.

tyrosine kinase." By way of comparison, claim 1 of the present application recites, "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase."

Claim 1 is unquestionably of similar scope and wording to claim 31 as originally filed. Whereas original claim 31 was directed to "a ligand," claim 1 is directed to "a purified and isolated polypeptide." However, the 1994 priority application clearly states that the ligand of the invention is a purified protein. (See, e.g., preliminary amendment to U.S.S.N. '08/340,011 dated November 14, 1994, at p. 15: "The purified biologically active ligand protein") Whereas original claim 31 was directed to binding to Flt4 receptor tyrosine kinase, claim 1 clarifies that the ligand binds to *the extracellular domain* of Flt4. However, the 1994 priority application clearly states that the ligand protein binds to the *Flt4 extracellular domain*. (See, e.g., preliminary amendment to U.S.S.N. '08/340,011 dated November 14, 1994, at p. 11: "The above experiments prove that the ligand binds to the recombinant FLT4 EC domain.") Finally, original claim 31 recites that the ligand "specifically binds" whereas claim 1 is directed to "high affinity" binding. However, this difference merely adopts preferred claim language suggested by the examiner in the course of prosecution. Thus, claim 1 is unquestionably of similar scope and wording to an original claim of the '011 patent application. (See the Heldin declaration at ¶ 6.) Accordingly, original claims in the '011 patent application provide written description support for claim 1 of the present patent application. See *In re Koller*, 204 U.S.P.Q. at 706.

Claim 19, which depends from claim 1 and recites that the polypeptide further comprises a detectable label, finds written description support in original claim 33 of the 1994 priority application. See *In re Koller*, 204 U.S.P.Q. at 706; see also the Heldin declaration at ¶ 7.

Claim 17 (which depended from claim 1 but has been rewritten as an independent claim incorporating the limitations of claim 1) is similar to claim 1 and additionally recites that the polypeptide is purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, the cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity

purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase. These additional limitations find explicit written description support in the 1994 priority application in Examples 12 and 15: Example 12 teaches that conditioned media from the PC-3 prostatic adenocarcinoma cell line (ATCC CRL 1435) produces a soluble ligand for Flt4 that binds to recombinant Flt4 extracellular domain and that can be purified using the Flt4 EC domain in affinity chromatography; Example 15 describes such affinity chromatography. (See the preliminary amendment to the '011 application at pp. 8-11 and 15.) Thus, claim 17 finds written description support in the original claims of the 1994 priority application coupled with the written description provided in Examples 12 and 15. (See the Heldin declaration at ¶ 8.)

Claim 21 as amended is identical to claim 19 except that claim 21 depends from claim 17. Thus, claim 21 finds written description support in the 1994 priority application for the reasons outlined above with respect to claims 17 and 19.

Claim 14 recites "A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase." Written description support for "a purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase" is discussed above in relation to claim 1. Example 12 in the 1994 priority application teaches that the Flt4 polypeptide ligand stimulates Flt4 tyrosine phosphorylation in mammalian cells that express Flt4 receptor tyrosine kinase. (See the preliminary amendment to the '011 application at pp. 8-11; see also p. 6 ("In a preferred embodiment of the invention, conditioned medium from the PC-3 cell line comprises a protein or fragment thereof, which is capable of stimulating the Flt4 receptor") Thus, claim 14 finds written description support in the original claims of the '011 application coupled with the written description provided in Example 12. (See the Heldin declaration at ¶ 9.)

Claims 13 depends from claim 1 and recites that the polypeptide has an apparent molecular weight of approximately 23 kD as assessed by SDS-

PAGE under reducing conditions. The approximate 23 kD molecular weight is an *inherent property* of the Flt4 ligand that the '011 application teaches one how to purify from the PC-3 conditioned medium. (See the Heldin declaration at ¶¶ 10 and 11.A.) As such, the inclusion of this property in the present application and in claim 13 does not deprive claim 13 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*, 33 U.S.P.Q.2d 1274, 1276 (Fed. Cir. 1995) ("A claim in a CIP application is entitled to the filing date of the parent application when the claimed invention is described in the parent specification in a manner that satisfies, inter alia, the description requirement of 35 U.S.C. §112. . . . [T]he later explicit description of an inherent property does not deprive the product of the benefit of the filing date of the earlier application."); *Ex parte Yamaguchi*, 6 U.S.P.Q.2d 1805, 1807 (PTO Bd. App. 1987) (Claim to compound characterized by a particular x-ray diffraction spectrum has written description support in earlier application that teaches the compound, notwithstanding the absence of any teaching of the x-ray diffraction pattern in the earlier application, because a compound and all of its properties are inseparable); see also *Application of Davies*, 177 U.S.P.Q. 381, 385, 475 F.2d 667, 671-72 (C.C.P.A. 1973) ("[W]e see no impediment to the present applicants' refiling their application and incorporating a discussion of the allegedly unobvious properties [of the invention] while retaining the effective date of the application involved here through §120.")

Claim 15 depends from claim 14 and further recites that the polypeptide "comprises an amino acid sequence set forth in SEQ ID NO: 13." This partial amino acid sequence is an *inherent property* of an Flt4 ligand that the 1994 priority application teaches one how to purify from the PC-3 conditioned medium. (See the present application at p. 19, lines 9-19 (teaching that Flt4 ligand affinity purified from PC-3 medium has an amino terminal amino acid sequence set forth in SEQ ID NO: 13); see also the Heldin declaration at ¶¶ 10 and 11.B.) As such, the inclusion of this property in the present application and in claim 15 does not deprive claim 15 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*; *Ex parte Yamaguchi*; and *Application of Davies, supra*.

Claim 16 (which depended from claim 13 but has been rewritten in independent form) additionally recites amino terminal amino acid sequence information of the claimed polypeptide. The recited amino acid sequence is an *inherent property* of the Flt4 ligand that the 1994 priority application teaches one how to purify from PC-3 conditioned medium.³ (See the Heldin declaration at ¶¶ 10 and 11.C.) As such, the inclusion of this property in the present application and in claim 16 does not deprive claim 16 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*; *Ex parte Yamaguchi*; and *Application of Davies, supra*.

New claim 28 is identical to claim 19 except that claim 28 depends from claim 16. Thus, claim 28 finds written description support in the 1994 priority application for the reasons outlined above with respect to claims 16 and 19.

Claim 23 depends from claim 14 and further recites that the polypeptide has "an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions." This molecular weight limitation is an *inherent property* of an Flt4 ligand that the 1994 priority application teaches one how to purify from the PC-3 conditioned medium, as discussed above with respect to claim 13. (See also the Heldin declaration at ¶¶ 10 and 11.D.) As such, the inclusion of this property in the present application and in claim 23 does not deprive claim 23 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*; *Ex parte Yamaguchi*; and *Application of Davies, supra*.

Moreover, the foregoing is not intended to be a complete list of those claims which find written description support in the specification. See the Heldin declaration at ¶11.)

³ Moreover, the 1994 priority application teaches to determine the amino terminal amino acid sequence. (See preliminary amendment to '011 application at p. 15 (Example 15).)

C. The Patent Office's reliance on the *Fiers* case is improper.

In dismissing the applicants' priority claim on written description grounds, the Patent Office relied upon the Federal Circuit's decision in *Fiers v. Revel*, 25 U.S.P.Q.2d 1601, 1606 (Fed. Cir. 1993). (Official action at pp. 2 and 3.) However, the *Fiers* opinion was rendered on its own distinct set of facts, and was rendered in the context of the state of the art in 1979-81 (i.e., about 13-15 years prior to the applicants' 1994 filing date). Since the issue of written description is factual in nature, *In re Alton, supra*, 37 U.S.P.Q.2d at 1580, the examiner's reliance upon a legal opinion rendered on different facts, and in a much earlier period of the art of molecular biology, is highly suspect from the outset.

1. The present application is distinguishable from the facts of the *Fiers* case because the present application teaches a method of preparing the claimed protein as a natural isolate.

The *Fiers* opinion was based on the premise that a written description of a DNA invention requires the same degree of specificity as a conception of a DNA invention. *Fiers*, 25 U.S.P.Q.2d at 1606. Citing its earlier opinion in *Amgen v. Chugai Pharmaceutical Co.*, 18 U.S.P.Q.2d 1016 (Fed. Cir. 1991), the Court acknowledged that conception of a DNA can occur where one is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. *Fiers*, 25 U.S.P.Q.2d at 1604. In the present case, the 1994 priority application is able to define the Flt4 ligand protein by a method of preparation (e.g., affinity purification using the Flt4 extracellular domain) and by chemical characteristics (e.g., a polypeptide that is capable of stimulating the Flt4 receptor and regulating vascular endothelial cells). Thus, under the standards articulated in the *Fiers* and *Amgen* cases for DNA inventions, the 1994 priority application contains a written description of the Flt4 ligand protein invention claimed herein.

2. The present application is distinguishable from *Fiers* because the invention presently claimed pertains to a purified protein.

In *Fiers*, the Federal Circuit rendered an opinion as to that which is required under §112, first paragraph, for an adequate written description of a DNA invention. The invention claimed in the present application is not a DNA invention;⁴ the invention pertains to a purified protein, and the issue concerns whether a priority application contains a sufficient written description of that protein invention. The examiner has failed to articulate why a factual determination in *Fiers* pertaining to a DNA invention is relevant to a factual determination pertaining to a protein invention in the present case.⁵ Accordingly, the examiner has failed to meet his burden of establishing a *prima facie* case of lack of written description.

Since the *Fiers* holding is distinguishable on its facts and also was rendered in the context of the state of the art in 1979-81, i.e., about 13-15 years prior to the applicants' 1994 filing date, the *Fiers* opinion fails to support the examiner's written description objection.

- D. The right of priority has no bearing on the patentability of any claim at this time, and therefore, is an inappropriate subject for Patent Office determination.

The Manual of Patent Examining Procedure instructs that a priority determination should be made during *ex parte* prosecution *only* when an intervening reference is found, upon which a rejection under §102 or §103 would be made:

The only times during *ex parte* prosecution that the examiner considers the merits of an applicant's claim of

⁴ The Patent Office has deemed the DNAs taught in the application to constitute a distinct invention.

⁵ In this regard, the Patent Office's attention is directed to *Scripps Clinic v. Genentech Inc.*, 18 U.S.P.Q. 1001 (Fed. Cir. 1991), an opinion issued contemporaneously with the *Amgen* opinion and pertaining to a purified protein invention. The Patent at issue in the *Scripps* case (Reissue Patent No. 32,011) contained claims to a purified protein (Factor VIII:C) and to an affinity method of purifying the protein. No amino acid sequence description was required under §112, first paragraph, for the Patent Office to issue or to reissue this patent.

priority is when a reference is found with an effective date between the date of the foreign filing and the date of filing in the United States and when an interference situation is under consideration. If at the time of making an action the examiner has found such an intervening reference, he or she simply rejects whatever claims may be considered unpatentable thereover, without paying any attention to the priority date

(M.P.E.P. (6th Ed., Rev. 2) §201.15.)

The outstanding final action constitutes the first time that the Patent Office has raised its written description objection as a basis for refusing to afford priority to the '011 application.⁶ However, there are no prior art rejections based upon intervening references in the outstanding action. Accordingly, under the Patent Office's own procedures, it was inappropriate to consider the merits of the priority claim in the official action.

E. Conditional Petition to Reverse or Withdrawn Adverse Priority Determination.

Should the examiner refuse to reverse or withdraw the adverse priority determination that was made for the first time in the final official action, the applicants hereby petition the commissioner to reverse this determination as improper, or, in the alternative, to withdraw this determination as premature and expunge from the file all mention of this premature determination. The facts in support of reversal of the priority determination are provided in parts A-C, above, and in the Declaration of Dr. Heldin filed herewith. The facts in support of withdrawal of the premature determination are provided in part D, above. In the event of withdrawal, the applicants respectfully submit that all mention of the priority determination in the final official action and this submission by the applicants should be expunged from the file, so as not to taint the file history of the eventual patent in a manner adverse to the applicants.

The priority issue is properly the subject of a petition because the priority determination is not pertinent to any rejection and, therefore, is not

⁶ See note 1, *supra*.

subject to review by the Board of Patent Appeals and Interferences. See M.P.E.P. §706.01.

The applicants hereby authorize the commissioner to charge any necessary petition fee associated with this conditional petition to Deposit Account No. 13-2855. This petition has been timely filed within two months of the mailing of the final official action that contains the adverse priority determination at issue.

IV. The amendments to claim 8 render moot the rejection of claims 8-9 and 19-20.

In paragraph 9 of the outstanding official action, the examiner rejected claims 8-9 and 20-22 under 35 U.S.C. § 101, asserting that these claims read on a product of nature, because claim 8 fails to recite a "purified and isolated" polypeptide. (Office action at p. 5.)

In response, the applicants have amended claim 8 to recite, "A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding." Thus, amended claim 8 does not read on a product of nature, rendering the rejection of claim 8 (and claims 9 and 19-20 which depend therefrom) moot. Since this amendment adopts a suggestion of the Patent Office and removes an issue for appeal, entry of the amendment and withdrawal of the rejection is respectfully requested.

V. The amendments to claims 16 and 17 place these claims in condition for allowance.

In paragraph 15 of the outstanding action, the Patent Office objected to claims 16 and 17 as being dependent upon a rejected base claim, but indicated that these claims would be allowable if rewritten in independent form. (Office action at p. 11.) In response, the applicants have rewritten claims 16 and 17 in independent form, incorporating all of the limitation of the base claim and any intervening claims. Accordingly, claim 16 and 17 are now in condition for allowance.

- VI. The amendments to claims 21, 22, and 25 place these claims in condition for allowance; and new claim 28 is in condition for allowance.

Claims 21, 22, and 25 have been amended to depend from and further limit claims 16 and 17. New claim 28 is identical to claim 21 and depends from claim 16. Because the subject matter of claims 16 and 17 has been deemed allowable, the amendment of claims 21, 22, and 25 (and addition of claim 28) to depend from claims 16 and 17 also places these claims in condition for allowance. Accordingly, entry of these amendments and allowance of claims 21, 22, 25, and 28 is respectfully requested.

- VII. The Patent Office's rejections of claims 1, 8, 9, 13-15, and 19-25 under §112, first paragraph, for lack of enablement improperly ignore both guidance provided in the specification and the skill of those in the art.

In paragraphs 10-13 of the official action, the examiner articulated his basis for maintaining rejections of claims 1, 8, 9, 13-15, and 19-25 under §112, first paragraph, for lack of enablement. The Patent Office admits that fragments of the protein of SEQ ID NO: 33 can be made, but asserts that undue experimentation would be required to screen all fragments of SEQ ID NO: 33 to determine which fragments bind the receptor:

The examiner argues that the claim limitation of binding the receptor must be met by testing all fragments encompassed by the claims, which in this case are not limited in any way.

(official action at p. 7.)⁷

The Patent Office's insistence that it is necessary to test all fragments of SEQ ID NO: 33 ignores the scientific ability of one of ordinary skill in the art. Importantly, one of ordinary skill in the art would not conduct experimentation by haphazardly making all of the possible fragments of SEQ ID NO: 33 and testing their ability to bind the receptor. An artisan of ordinary skill understands that each fragment that is screened provides guidance as to that

⁷ Claim 8 encompasses only polypeptides which are capable of binding the Flt4 receptor. To the extent that the examiner has interpreted claim 8 (or similarly limited claims) to "encompass" all fragments of SEQ ID NO: 33, the examiner has ignored a limitation of claim 8 and thereby erroneously construed the claim.

portion of SEQ ID NO: 33 that is effective for binding, and that portion which is not.⁸ An artisan of ordinary skill also understands techniques for accelerating a screening process,⁹ and techniques for screening multiple polypeptides *simultaneously*. Thus, the examiner's reasoning greatly overstates both the quantity and the nature of the experimentation required to practice the invention as claimed.

In this regard, the application provides explicit guidance for screening fragments of SEQ ID NO: 33 to determine a portion effective to permit Flt4 binding. Although SEQ ID NO: 33 contains 350 amino acids, the specification provides guidance that the region critical for receptor activation is contained within its first approximately 180 amino acid residues.

By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin, et al., Growth Factors 8, 245-252, 1993) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues. (Specification, pp. 27-28.)

To determine which fragments contain a sufficient portion of SEQ ID NO: 33 to permit binding, the specification also outlines a specific protocol. The specification teaches one skilled in the art to (a) generate progressive deletion products of the Flt4 ligand cDNA; (b) express these modified cDNAs; and (c) assay the resulting truncated protein forms, e.g., by studying their ability to induce Flt4 autophosphorylation. (Specification at, e.g., p. 27, lines 23-29.) These teachings serve to both provide guidance for predicting the portions of

⁸ For example, a determination that a polypeptide comprising residues 34-180 of SEQ ID NO: 33 is effective to permit binding to Flt4 and that a polypeptide comprising residues 181-350 is ineffective to permit binding would provide significant guidance as to that portion of SEQ ID NO: 33 to further screen for effective fragments. Thus, the assertion that it would be necessary to screen "all" fragments of SEQ ID NO: 33 to practice the claimed invention relies upon the false assumption that individual screening assays will be performed without knowledge gained from prior screenings.

⁹ For example, it is within the skill of the art to synthesize spaced deletion mutants (e.g., residues 34-350, 34-330, 34-310, etc.) from SEQ ID NO: 33, rather than successive deletion mutants (34-350, 34-349, 34-348 . . .), to more rapidly identify effective portions for binding Flt4.

SEQ ID NO: 33 that are effective to permit Flt4 binding; and (2) reduce the amount of experimentation required to determine the minimum portion of SEQ ID NO: 33 that is critical for receptor binding.


Moreover, as explained above, it is within the skill of the art to synthesize deletion mutants of SEQ ID NO: 33 that have been spaced intermittently (e.g., residues 34-180, 34-160, 34-140, 34-120, etc.), rather than synthesize every possible successive deletion mutant (34-180, 34-179, 34-178, 34-177 . . .), to more rapidly identify effective portions for binding Flt4. Furthermore, the skilled artisan is capable of synthesizing and screening several such deletion fragments simultaneously, in parallel experiments. Thus, the examiner's assertions that it is necessary to screen every fragment of SEQ ID NO: 33, that the specification lacks guidance, and that the amount of screening required constitutes undue experimentation is improper. See *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988) ("Enablement is not precluded by the necessity for some experimentation such as routine screening. . . . The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.")

VIII. Summary

For the foregoing reasons, the applicants respectfully request reconsideration, withdrawal of all claim rejections and objections to the specification, withdrawal of the notation that no claims are afforded priority to the parent application, and allowance of claims 1-2, 8-9, 12-17, and 19-28.

Respectfully submitted,

June 11, 1997


James P. Zeller
Registration No. 28,491
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

Appendix of claims

1. (Twice amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase.

2. (Amended) A purified and isolated polypeptide comprising an amino acid sequence shown in SEQ ID NO: 33.

8. (Three times amended) A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding.

9. (Twice amended) A polypeptide according to claim 8 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

12. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

13. A polypeptide according to claim 1 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

14. (Amended) A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

15. A purified and isolated polypeptide according to claim 14, said polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13.

16. (Amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

17. (Twice amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, said polypeptide being purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

19. A polypeptide according to claim 1 further comprising a detectable label.

20. A polypeptide according to claim 8 which is capable of binding the extracellular domain of Flt4 receptor tyrosine kinase with high affinity and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

21. (Amended) A polypeptide according to claim 17 further comprising a detectable label.

22. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 17 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

23. A polypeptide according to claim 14 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

24. A polypeptide according to claim 14 comprising a portion of SEQ ID NO: 33 effective to permit binding to Flt4 receptor tyrosine kinase and stimulation of Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

25. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 16 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

26. A polypeptide according to claim 8 wherein said portion of SEQ ID NO: 33 effective to permit such binding is a continuous portion of SEQ ID NO: 33 within amino acids 1-180 of SEQ ID NO: 33.

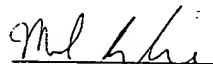
27. A polypeptide according to claim 8 wherein the amino terminus of said portion effective to permit such binding corresponds with position 34 of SEQ ID NO: 33.

28. A polypeptide according to claim 16 further comprising a detectable label.



PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:)	"EXPRESS MAIL"
Alitalo et al.)	Mailing label No. EM099827086US
Serial No.: 08/510,133)	Date of Deposit: June 11, 1997
Filed: August 1, 1995)	I hereby certify that this paper and the documents
For: RECEPTOR LIGAND)	referred to as enclosed herewith are being
Group Art Unit: 1814)	deposited with the United States Postal Service
Examiner: Lathrop, B.)	"EXPRESS MAIL POST OFFICE TO ADDRESSEE"
)	service under 37 CFR §1.10 on the date indicated
)	above and is addressed to the Assistant
)	Commissioner for Patents,
)	Washington, D.C. 20231.
)	
)	Mark Bonadonna

Declaration of Carl-Henrik Heldin
Pursuant to 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

RECEIVED
JUN 16 1997
GROUP 1800

Sir:

I, Carl-Henrik Heldin, hereby state as follows:

1. I am Director and member of the Uppsala Branch of Growth Regulation of the Ludwig Institute of Cancer Research (the Ludwig Institute) in Uppsala, Sweden. My curriculum vitae is attached hereto as Exhibit A.

2. I understand that on 01 August 1995, Dr. Kari Alitalo and Dr. Vladimir Joukov (as inventors) filed U.S. Patent Application Serial No. 08/510,133 (hereinafter "the 1995 application"), directed to a polypeptide ligand for Flt4 receptor tyrosine kinase; fragments thereof; a polynucleotide encoding the ligand; vectors and host cells comprising the polynucleotide; and

antibodies reactive with the ligand. I understand that the Ludwig Institute now has an ownership interest in this application.

3. I further understand that, during examination of the 1995 application by the U.S. Patent and Trademark Office (the Patent Office), the examiner has taken the position that U.S. Patent Application Serial No. 08/340,011, filed on 14 November 1994 ("the 1994 application") does not contain a written description of the polypeptide invention that is being claimed in the 1995 application. I have been asked by the Ludwig Institute to review the 1994 and 1995 applications and to provide a factual analysis of whether the 1994 application contains a written description of the invention that is being claimed in the 1995 application.

4. I understand that the claims in a patent application are the portion of a patent application that defines the invention for which patent applicants seek patent protection. I further understand that patent applications are written for the practitioner of ordinary skill in the pertinent scientific field. In the scientific specialties or subdisciplines which fall within the general category of "cellular and molecular biology," the reader of ordinary skill in 1994 and 1995 (hereinafter "the reader") would have had at least a medical or doctorate degree and probably at least some post-doctoral research experience.

5. To perform this analysis, I have reviewed and understand the contents of the 1994 application. This review included the document titled "Preliminary Amendment" that was filed on 14 November 1994 (hereinafter "the Preliminary Amendment"). I understand that pages 2-19 of the Preliminary Amendment contain text, examples, and claims which are considered part of the 1994 application. I also have reviewed and understand the contents of the 1995 application, including the claims thereof. Exhibit B hereto contains the pending claims of the 1995 application, with claim amendments that the Applicants intend to file with the Patent Office contemporaneously with this declaration.

6. From the facts summarized below, I conclude that the subject matter of claim 1 of the 1995 application is described in the 1994 application in a manner which apprises the reader that the inventors had possession of a concept of what is claimed. Stated another way, the 1994 application reasonably conveys to me that the inventors had possession of the subject matter of claim 1 of the 1995 application, at the time that the 1994 application was filed:

A. Claim 31 of the 1994 application recites, "A ligand which specifically binds to an FLT-4 receptor tyrosine kinase." (See the Preliminary Amendment at p. 18.) Since claims in a patent application define the invention for which patent applicants seek patent protection, it is absolutely clear to me that the inventors considered an Flt4 ligand to be an aspect of their invention.

B. Claim 1 of the 1995 application recites, "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase." Thus, whereas claim 31 of the 1994 application was directed to "a ligand," claim 1 of the 1995 application is directed to "a purified and isolated polypeptide." However, the 1994 application clearly states that the ligand of the invention is a purified protein. (See, e.g., the Preliminary Amendment at p. 15 ("The purified biologically active ligand protein"); see also p. 6 ("In a preferred embodiment of the invention, conditioned medium from the PC-3 cell line comprises a protein or a fragment thereof, which is capable of stimulating the FLT4 receptor....").) Therefore, the "purified and isolated polypeptide" recitations of claim 1 are described in the 1994 application.

C. Whereas claim 31 of the 1994 application was directed to binding "to an FLT-4 receptor tyrosine kinase," claim 1 of the 1995 application specifies that the ligand binds "to the extracellular domain of Flt4 receptor tyrosine kinase." However, the 1994 application clearly states that the ligand protein binds to the Flt4 extracellular domain. (See, e.g., the Preliminary Amendment at p. 11: "The above experiments prove that the ligand binds to the recombinant FLT4 EC [extracellular] domain.")

Therefore, the recitations in claim 1 regarding binding to the Flt4 extracellular domain are described in the 1994 application.

D. Claim 31 of the 1994 application recites that the ligand "specifically binds," whereas claim 1 of the 1995 application is directed to "high affinity" binding. However, the reader would have understood that the "ligand" that "specifically binds" to Flt4 receptor was a high affinity binding partner. For example, the teaching in the 1994 application to purify the ligand using the recombinant FLT4 EC domain in affinity chromatography (see, e.g., the Preliminary Amendment at p. 11 and Example 15) apprises the reader that the ligand is thought to be a high affinity ligand.

Thus, I conclude that the subject matter of claim 1 of the 1995 application is described in claim 31, at pp. 11 and 15 of the Preliminary Amendment, and elsewhere in the 1994 application.

7. I conclude that the subject matter of claim 19 of the 1995 application is described in the 1994 application in a manner which apprises the reader that the inventors had possession of a concept of what is claimed. Claim 19 of the 1995 application is directed to the polypeptide having all of the features recited in claim 1 of the 1995 application, and "further comprising a detectable label." Thus, the only aspect of claim 19 not already discussed above (in paragraph 6) is the inclusion of a detectable label. However, claim 33 of the 1994 patent application recites, "The ligand according to claim 31 comprising a label." (See the Preliminary Amendment at p. 18.) Since claims in a patent application define the invention for which patent applicants seek patent protection, it is absolutely clear to the reader from claims 31 and 33 of the 1994 application that the inventors considered an Flt4 ligand which includes a label to be an aspect of their invention. The property of being "detectable" is understood in the art to be inherent in a "label." (The purpose of a label is to provide a means for detecting the substance that carries the label.) Moreover, this understanding is confirmed by claims 34 and 35 of the 1994 application, which are directed to methods which involve "detecting" the labeled ligand.

(See the Preliminary Amendment at p. 19.) Thus, the 1994 application reasonably conveys to me that the inventors had possession of the subject matter of claim 19 of the 1995 application, at the time that the 1994 application was filed.

8. I conclude that the subject matter of claim 17 of the 1995 application is described in the 1994 application in a manner which apprises the reader that the inventors had possession of a concept of what is claimed. Claim 17 is similar to claim 1 of the 1995 application and additionally recites that the polypeptide is "purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase." These additional properties are explicitly described in the 1994 application in Examples 12 and 15: Example 12 teaches that conditioned media from the PC-3 prostatic adenocarcinoma cell line (ATCC CRL 1435) produces a soluble ligand for Flt4 that binds to recombinant Flt4 extracellular domain and that can be purified using the Flt4 EC domain in affinity chromatography (see the Preliminary Amendment at pp. 8-11); Example 15 describes such affinity chromatography. (*Id.* at p. 15.) Thus, the 1994 application reasonably conveys to me that the inventors had possession of the subject matter of claim 17 of the 1995 application, at the time that the 1994 application was filed.

9. I conclude that the subject matter of claim 14 of the 1995 application is described in the 1994 application in a manner which apprises the reader that the inventors had possession of a concept of what is claimed. Claim 14 recites, "A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase." Descriptive support in the 1994 application for "a purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase" is discussed above with respect to claim 1. (See paragraph 6, above.) Example 12 in the 1994 application teaches that the

Flt4 polypeptide ligand stimulates Flt4 tyrosine phosphorylation in mammalian cells that express Flt4 receptor tyrosine kinase. (See the Preliminary Amendment at pp. 8-11; see also p. 6 ("In a preferred embodiment of the invention, conditioned medium from the PC-3 cell line comprises a protein or fragment thereof, which is capable of stimulating the Flt4 receptor....").) Thus, the 1994 application reasonably conveys to me that the inventors had possession of the subject matter of claim 14 of the 1995 application, at the time that the 1994 application was filed.

10. It is fundamental biochemistry that polypeptides are organic chemical compounds, albeit sometimes large and complex ones. Like all organic chemical compounds, polypeptides may be characterized by any of several inherent physical properties, such as molecular formula and molecular weight. Such physical properties are *inherent* characteristics of organic molecules in that they are intrinsic properties of the molecules. Because polypeptides are themselves composed of covalently-bonded chains of smaller organic moieties called amino acids (of which there are about 20 naturally occurring), it is conventional to express the molecular formula of polypeptides as an amino acid sequence. The amino acid sequence of any polypeptide is an inherent property of that polypeptide.

11. Certain claims in the 1995 application recite subject matter that is described in the 1994 application, and also recite certain inherent properties of that subject matter.

A. For example, claims 13 recites a polypeptide having all of the characteristics described in claim 1 and further recites that the polypeptide has "an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions." The subject matter of claim 1 is described in the 1994 application. (See paragraph 6, above.) The approximate 23 kD molecular weight that is recited in claim 13 is an *inherent property* of an Flt4 ligand that the 1994 application teaches one how to purify from the PC-3 conditioned medium. (See the

1995 application at pp. 18-19 (teaching that the Flt4 ligand that was affinity purified from PC-3 medium had an apparent molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions).)

B. Claim 15 recites a polypeptide having all of the characteristics described in claim 14 and further recites that the polypeptide comprises "an amino acid sequence set forth in SEQ ID NO: 13." The partial amino acid sequence set forth in SEQ ID NO: 13 of the 1995 application is an *inherent property* of an Flt4 ligand that the 1994 application teaches one how to purify from the PC-3 conditioned medium. (See the 1995 application at p. 19, lines 9-19 (teaching that Flt4 ligand that was affinity purified from PC-3 medium had an amino terminal amino acid sequence set forth in SEQ ID NO: 13).)

C. Claim 16 recites a polypeptide having all of the characteristics described in claim 13 and further recites that amino acids 2 through 18 of the polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13. Thus, for the reasons described above with respect to claims 13 and 15 (in Parts A and B), the features recited in claim 16 are inherent properties of an Flt4 ligand that the 1994 application teaches one how to purify from PC-3 conditioned medium.

D. Claim 23 recites a polypeptide having all of the characteristics described in claim 14 and further recites that the polypeptide has "an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions." The subject matter of claim 14 is described in the 1994 application. (See paragraph 9, above.) The approximate 23 kD molecular weight further recited in claim 23 is an *inherent property* of an Flt4 ligand that the 1994 application teaches one how to purify from a PC-3 conditioned medium, as discussed in Part A above with respect to claim 13.

The foregoing is not intended to constitute a complete list of those claims which recite inherent properties of an Flt4 ligand described in the 1994 application. For example, the 1995 application teaches a cDNA nucleotide sequence and a

deduced amino acid sequence of a precursor of a 23 kD Flt4 ligand taught in the 1994 application. (See, e.g., 1995 application at p. 5, lines 13-20.) Thus, according to the 1995 application, an inherent property of an Flt4 ligand taught in the 1994 application is that the ligand has an amino acid sequence comprising a portion of SEQ ID NO: 33 that is effective to permit binding to Flt4 receptor tyrosine kinase and stimulate phosphorylation thereof. These properties are recited in several claims of the 1995 application other than those specifically discussed above.

12. The 1994 application teaches the reader how to purify and isolate an Flt4 ligand from conditioned medium of a prostatic cell line, using an affinity chromatography method:

A. Example 12 in the 1994 application teaches the reader how to prepare a conditioned medium comprising an Flt4 ligand by culturing the PC-3 prostatic adenocarcinoma cell line (ATCC CRL 1435) for seven days in F12 medium in the absence of serum, and then clarifying the medium by centrifugation. (See the Preliminary amendment at p. 8.) Example 4 in the 1995 application contains a similar teaching.

B. Example 12 in the 1994 application contains experimental data proving that the PC-3 conditioned medium contains a ligand that is capable of stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, in cells expressing Flt4 receptor tyrosine kinase. (See the Preliminary Amendment at pp. 8-11.) Moreover, Example 12 in the 1994 application characterizes the Flt4 ligand as a moiety of at least 10,000 molecular weight, and teaches that the medium can be concentrated with a commercially available Centricon-10 concentrator, in order to increase Flt4 ligand activity. (Preliminary Amendment at p. 11.)

C. Example 12 further teaches that treatment of the concentrated PC-3 conditioned medium with Flt4 extracellular domain fragment coupled to Sepharose beads (a solid support) will remove the Flt4 ligand from the conditioned medium. (See the Preliminary Amendment at p. 11 (pretreatment of the concentrated conditioned medium with Flt4EC-

Sepharose abolished the ability of the conditioned medium to stimulate Flt4 phosphorylation).) This teaching provides direct evidence that the ligand of the invention binds to the extracellular domain of Flt4, and thus that the ligand can be purified using the recombinant Flt4 extracellular domain in affinity chromatography.

D. Example 14 of the 1994 application teaches how to make recombinant Flt4 extracellular domain protein to use in an affinity chromatography matrix to purify the Flt4 ligand. (See, e.g., the Preliminary Amendment at p. 13.) Example 3 of the 1995 application contains a similar teaching.

E. Example 15 of the 1994 application teaches how to purify the Flt4 ligand using affinity chromatography procedures. In one of the procedures, the affinity matrix is Flt4 extracellular domain protein that has been cross-linked to CNBr-activated Sepharose 4B (a commercially available solid support that is useful for generating an affinity matrix). The reader in 1994 would have understood that affinity purification involves contacting the ligand-containing solution with the affinity matrix to permit binding between the ligand and the affinity matrix; washing the affinity matrix to remove unbound impurities; and eluting the ligand with an eluting solution. Typically, all fractions removed from the matrix (wash fractions and elution fractions) are assayed to determine in which fractions the ligand of interest has eluted. Example 15 of the 1994 application teaches to use an Flt4 phosphorylation assay to determine which chromatography fractions contained the Flt4 ligand. (See the Preliminary Amendment at p. 15.) The phosphate buffered saline and phosphate buffer wash solutions that were actually used (see the 1995 application at Example 5, p. 18) are typical wash solutions for a protein affinity chromatography. Moreover, the reader would have known that varying parameters such as ionic strength, pH, and the hydrophilic/hydrophobic character of the eluting solutions are conventional methods for eluting a compound of interest from an affinity chromatography column. Thus, the details in Example 15 of the 1994

application enable the reader to purify the Flt4 ligand by affinity chromatography.

F. The 1994 application teaches to subject the Flt4 ligand material that is eluted from the affinity column to further purification, using ion exchange and reverse-phase high pressure chromatography and SDS-polyacrylamide gel electrophoresis. (See the Preliminary Amendment at p. 15.) While the reader would have been able to perform all three of these conventional techniques, it is clear from the results reported in the 1995 application that sufficiently pure Flt4 ligand is obtained (e.g., sufficiently pure for amino acid sequencing) simply with the affinity purification followed by the SDS-PAGE procedure. (See the 1995 application at Example 5, pp. 17-19.) The ion exchange and reverse-phase chromatography were unnecessary.

Thus, the 1994 application teaches the reader how to purify and isolate an Flt4 ligand. The 1995 application describes results of such a purification, thereby demonstrating that the affinity purification method taught in the 1994 application works successfully.

13. The 1994 application teaches several uses for purified Flt4 ligand. These uses include:

A. Isolating a gene encoding the Flt4 ligand by microsequencing the purified ligand to determine a partial amino acid sequence; generating oligonucleotide probes based on the amino acid sequence (See the Preliminary Amendment, Example 15, p. 15; and Example 12, pp. 11-12); using the oligonucleotides as hybridization probes or PCR primers to isolate a ligand-encoding cDNA clone from a cDNA library generated from PC-3 poly-A RNA (*id.*, Examples 16 and 17A, p. 16);

B. use in an assay system to screen for inhibitors of Flt4 ligand/Flt4 receptor tyrosine kinase interaction (Preliminary Amendment at pp. 6 and 7);

C. regulating the growth, differentiation, and functions of endothelial cells, particularly lymphatic endothelia (Preliminary Amendment at p. 7);

D. generating antibodies against the Flt4 ligand (Preliminary Amendment at p. 7);

E. use in an assay to detect the presence of FLT4 receptor tyrosine kinase (see the Preliminary Amendment at p. 19, claim 35); and

F. use in an assay to detect endothelial cell proliferation (*id.*, claim 34).

14. With respect to my conclusions in paragraphs 6-13, above, I believe that the reader of ordinary skill in the field in 1994 who reviewed the 1994 application would have reached the same conclusions: that the inventors had possession of a concept of what is now being claimed in the present application. Stated another way, the priority application reasonably would have conveyed to the skilled artisan that the inventors had possession of the Flt4 ligand invention recited in claims of the 1995 application, of how to purify the ligand, and how to use the ligand.

15. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

June 4, 1997
Date


Carl-Henrik Heldin

CURRICULUM VITAE

97-05-28

Name: Carl-Henrik Heldin

Present appointment: Director
Ludwig Institute for Cancer Research
(Uppsala Branch)
Biomedical Center, Box 595
S-751 24 Uppsala, Sweden
Phone: +46-18-160401
Fax: +46-18-160420
E-mail: C-H.Heldin@LICR.uu.se

Home address: Hesselmans väg 35
S-752 63 Uppsala, Sweden
Phone: +46-18-463213

Date and place of birth: August 9, 1952, Växjö, Sweden

Nationality: Swedish

Marital status: Married, two children, born 1982 and 1988

University education:

1971-1975	First four years of Medical School completed (University of Uppsala)
1972-1981	Bachelor of Science (Mathematics 1 1/2 year, Numeric analysis 1/2 year, Psychology 1/2 year, Greek 1/2 year) completed July 28, 1981 (University of Uppsala)
1975-1980	Thesis work at Department of Medical and Physiological Chemistry (University of Uppsala). Dissertation May 10, 1980. "Studies on growth factors for human cultured cells".

Academic positions:

1972-1974	Part time teaching positions at Depts of Anatomy, Medical and Physiological Chemistry, and Physiology (in total 200 hours)
75.07.01-80.03.31.	Graduate student scholarship at Dept of Medical and Physiological Chemistry combined with a part time teaching position (in total 1100 hours)
80.04.01-80.10.31	Research Assistant at Dept of Medical and Physiological Chemistry
81.01.01-81.03.31	Lecturer at Dept of Medical and Physiological Chemistry
81.07.01-83.12.31	Cancer Research Scholarship from the Swedish Cancer Society
84.01.01-85.12.31	Senior Scientist of the Swedish Cancer Society
86.01.01--	Director, Ludwig Institute for Cancer Research (Uppsala Branch)
92.08.01--	Professor in Molecular Cell Biology at the Medical Faculty of Uppsala University

Positions as physician:	75.06.23-75.08.16	Geriatric Clinic at Härnösand's Hospital
	79.06.25-79.08.01	Clinical Chemistry at Sundsvall's Hospital
Academic honours:	1981	Appointed docent in Medical and Physiological Chemistry at Uppsala University
	1986	Appointed adjunct professor in Medical and Physiological Chemistry at Uppsala University
	1989	Elected member of European Molecular Biology, Organization
	1991	Elected member of the Royal Swedish Academy of Sciences, Medical Class
Awards:	1984	The The Swedberg Prize, from The Swedish Biochemical Society
	1984	King Oscar II:s Prize, from the University of Uppsala (shared with K. Söderhäll)
	1984	The Alvarenga Prize, from the Swedish Medical Society (shared with B. Westermark)
	1984	The Thureus Prize, from the Royal Society of Sciences (Uppsala)
	1986	Anders Jahre's Medical Prize for Younger Scientists, from the University of Oslo (shared with B. Gustafsson)
	1989	Prix Antoine Lacassagne, from the French National Organization against Cancer (shared with D. Gospodarowicz)
	1989	K. Fernström's Medical Prize for Young Swedish Scientists, from the Medical Faculty of Uppsala University
	1990	The Jubilee Prize, from the Swedish Medical Society (shared with B. Westermark)
	1992	The EMBO Medal, from the EMBO Council
Thesis adviser:	1993	K. Fernström's Large Medical Prize for Nordic Scientists, from the Medical Faculty of Lund (shared with B. Westermark)
		Thesis adviser for 12 graduate students: Bo Ek (dissertation 1985), Ann Johnsson (1986), Lars Rönstrand (1989), Annet Hammacher (1989), Arne Ösman (1990), Kensuke Usuki (1992), Maria Andersson (1994), Flemming Vassbotn (1994), Anders Olofsson (1995), Peter Blume-Jensen (1995), Klaus Hansen (1996), Jan Saras (1997).
Assistant thesis adviser:		Assistant thesis adviser for 10 graduate students: Christer Betsholtz (1986), Monica Nistér (1987), Karin Mellström (1988), Ylva Paulsson (1988), Anders Tingström (1991), Anders Eriksson (1992), Anja Smits (1992), Monica Hermanson (1993), Petra Franzén (1995), Koutaro Yokote (1996).

Thesis examiner:	<p>Ylva Engström, Karolinska Institute, Stockholm, December 17, 1985. Eva Dafgård, Karolinska Institute, Stockholm, February 1, 1991. Kristian Helin, University of Copenhagen, Denmark, June 25, 1991. Eva Jacobson, University of Stockholm, May 9, 1994. Pia Ljungquist-Höddelius, University of Linköping, Sept. 30, 1994. Tim Wood, Karolinska Institute, Stockholm, December 19, 1996. Martin Ridderstråle, University of Lund, February 28, 1997. Mikael Rydén, Karolinska Institute, Stockholm, April 25, 1997. Lone Rønnev-Jessen, University of Copenhagen, May 16, 1997.</p>
Referee for professorships:	<p>Professor in Medical Biochemistry, Oulu, Finland, 1989. Professor in Cell Biology, Linköping, Sweden, 1992 Professor in Cell Biology/Physiology, Linköping, Sweden, 1992. Professor in Molecular Cell Biology, Helsinki, Finland, 1993. Professor in Molecular Cell Biology, Lund, Sweden, 1995.</p>
Scientific conferences:	<p>Participated in 119 scientific conferences outside Sweden between 1977 and 1995 (in 111 as an invited lecturer). Co-organized 8 international meetings or courses between 1987 and 1995.</p>
Scientific advisory and review committees:	<p>Member, Scientific Advisory Committee for Heinrich-Pette-Institut für Virologie, Hamburg, 1988-1995. Member, Coordinating Committee, European Science Foundation Network on Developmental Biology, 1989-1991. Member, Scientific Advisory Board, European Organization for Research and Treatment of Cancer, 1989-1991. Member, Priority Committee A, Swedish Cancer Society, 1989-1994. Vice Chairman, Priority Committee A and Member, Research Board, Swedish Cancer Society, 1995- Chairman, Priority Committee A2 and Member, Research Board, Swedish Cancer Society, 1997- Member, Scientific Advisory Committee, Danish Biotechnology Program, 1990-1995. Member, Scientific Review Committee, Differentiation programme, EMBL, 1991 and 1995. Member, Priority Committee Chemistry I, Swedish Medical Research Council, 1993-1994. Member, Scientific Review Committee, CRC Growth Factor Research Group, Oxford, 1993. Member, Expert Committee on Medical Bioscience, Foundation for Strategic Research, 1994-1996. Member, Scientific Review Committee, ICRF Laboratories, Institute of Molecular Medicine and Clinical Oncology Unit, Oxford, 1994. Member, International Advisory Board, The Haartman Institute, Helsinki, 1996- Member, Advisory Board, Division of Cancer Biology, Danish Cancer Society, 1996- Member, Scientific Review Committee, Biocenter, Oulu, 1996.</p>

Scientific award committees:	<p>Member, Sloan Prize Selection Committee, General Motors Cancer Research Awards, 1988-1989.</p> <p>Vice Chairman, Sloan Prize Selection Committee, General Motors Cancer Research Awards, 1989-1990.</p> <p>Member, Prix Antoine Lacassagne Selection Committee, French National Organisation against Cancer, 1992-1995.</p> <p>Deputy Member, Board of the Göran Gustafsson Foundation, 1994-</p>
Other committees:	<p>Member, Board of the Faculty of Medicine, Uppsala University, 1978-1980.</p> <p>Deputy Member, Board of the Faculty of Medicine, Uppsala University, 1993-</p> <p>Member, Research Committee, Faculty of Medicine, Uppsala University, 1993-</p> <p>Member, Committee to recommend organization of molecular biology at the University of Trondheim, Norway, 1995</p>
Associate editor:	<p>Growth Factors, 1988-</p> <p>Molecular Biology of the Cell (formerly Cell Regulation), 1989-</p> <p>Cancer Research, 1993-</p> <p>Genes to Cells, 1995-</p>
Editorial boards:	<p>European Journal of Biochemistry, 1987-1992</p> <p>In Vitro Cellular and Developmental Biology, 1988-</p> <p>Progress in Growth Factor Research, 1988-1995</p> <p>Biochemistry, 1989-1992</p> <p>Journal of Vascular Medicine and Biology, 1989-</p> <p>International Journal of Cancer, 1989-</p> <p>Oncogenes and Growth Factors Abstracts, 1989-</p> <p>EMBO Journal, 1990-1992</p> <p>European Journal of Cancer, 1990-</p> <p>Trends in Biological Sciences, 1990-</p> <p>Journal of Vascular Research, 1991-</p> <p>Pathogenesis, 1996-</p> <p>Journal of Cellular Physiology, 1996-</p> <p>Cytokine and Growth Factor Reviews, 1996-</p> <p>Journal of Cell Science, 1996-</p>

Carl-Henrik Heldin

RESEARCH ARTICLES

1997-05-28

1. Heldin, C.-H., Wasteson, Å., and Westermark, B. (1977). Partial purification and characterization of platelet factors stimulating the multiplication of normal human glial cells. *Exp. Cell Res.* 109, 429-437.
2. Wasteson, Å., Glimelius, B., Busch, C., Westermark, B., Heldin, C.-H., and Norling, B. (1977). Effect of a platelet endoglycosidase on cell surface associated heparan sulphate of human cultured endothelial and glial cells. *Thrombosis Res.* 11, 309-321.
3. Heldin, C.-H., Westermark, B., and Wasteson, Å. (1979). Platelet-derived growth factor: Purification and partial characterization. *Proc. Natl. Acad. Sci. USA* 76, 3722-3726.
4. Heldin, C.-H., Westermark, B., and Wasteson, Å. (1979). Desensitisation of cultured glial cells to epidermal growth factor by receptor down-regulation. *Nature* 282, 419-420.
5. Heldin, C.-H., Westermark, B., and Wasteson, Å. (1980). Chemical and biological properties of a growth factor from human-cultured osteosarcoma cells: Resemblance with platelet-derived growth factor. *J. Cell. Physiol.* 105, 235-246.
6. Oldberg, Å., Heldin, C.-H., Wasteson, Å., Busch, C., and Höök, M. (1980). Characterization of a platelet endoglycosidase degrading heparin-like polysaccharides. *Biochemistry* 19, 5755-5762.
7. Heldin, C.-H., Wasteson, Å., and Westermark, B. (1980). Growth of normal human glial cells in a defined medium containing platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA* 77, 6611-6615.
8. Heldin, C.-H., Westermark, B., and Wasteson, Å. (1981). Platelet-derived growth factor: Isolation by a large-scale procedure and analysis of subunit composition. *Biochem. J.* 193, 907-913.
9. Heldin, C.-H., Wasteson, Å., Fryklund, L., and Westermark, B. (1981). Somatomedin B: Mitogenic activity derived from contaminant epidermal growth factor. *Science* 213, 1122-1123.
10. Heldin, C.-H., Westermark, B., and Wasteson, Å. (1981). Demonstration of an antibody against platelet-derived growth factor. *Exp. Cell Res.* 136, 255-261.
11. Heldin, C.-H., Westermark, B., and Wasteson, Å. (1981). Specific receptors for platelet-derived growth factor on cells derived from connective tissue and glia. *Proc. Natl. Acad. Sci. USA* 78, 3664-3668.
12. Ek, B., Westermark, B., Wasteson, Å., and Heldin, C.-H. (1982). Stimulation of tyrosine-specific phosphorylation by platelet-derived growth factor. *Nature* 295, 419-420.
13. Heldin, C.-H., Wasteson, Å., and Westermark, B. (1982). Interaction of platelet-derived growth factor with its fibroblast receptor. Demonstration of ligand degradation and receptor modulation. *J. Biol. Chem.* 257, 4216-4221.
14. Johnsson, A., Heldin, C.-H., Westermark, B., and Wasteson, Å. (1982). Platelet-derived growth factor: Identification of constituent polypeptide chains. *Biochem. Biophys. Res. Commun.* 104, 66-74.

15. Ek, B., and Heldin, C.-H. (1982). Characterization of a tyrosine-specific kinase activity in human fibroblast membranes stimulated by platelet-derived growth factor. *J. Biol. Chem.* 257, 10486-10492.
16. Edlund, B., Heldin, C.-H., and Engström, L. (1982). Effect of chemical modification of a histidine and a lysine residue of pea seed nucleoside diphosphate kinase. *J. Med. Sci.* 87, 243-250.
17. Westermark, B., Magnusson, A., and Heldin, C.-H. (1982). Effect of epidermal growth factor on membrane motility and cell locomotion in cultures of human clonal glioma cells. *J. Neuroscience Res.* 8, 491-507.
18. King, G.L., Kahn, C.R., and Heldin, C.-H. (1983). Sharing of biological effect and receptors between guinea pig insulin and platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA* 80, 1308-1312.
19. Nilsson, J., Ksiazek, T., Heldin, C.-H., and Thyberg, J. (1983). Demonstration of stimulatory effects of platelet-derived growth factor on cultivated rat arterial smooth muscle cells: Differences between cells from young and adult animals. *Exp. Cell Res.* 145, 231-237.
20. Mellström, K., Höglund, A.-S., Nistér, M., Heldin, C.-H., Westermark, B., and Lindberg, U. (1983). The effect of platelet-derived growth factor on morphology and motility of human glial cells. *J. Musc. Res. Cell Motility* 4, 589-609.
21. Heldin, C.-H., Ek, B., and Rönnstrand, L. (1983). Characterization of the receptor for platelet-derived growth factor on human fibroblasts. Demonstration of an intimate relationship with a 185,000-dalton substrate for the platelet-derived growth factor-stimulated kinase. *J. Biol. Chem.* 258, 10054-10061.
22. Nilsson, J., Thyberg, J., Heldin, C.-H., Westermark, B., and Wasteson, Å. (1983). Surface binding and internalization of platelet-derived growth factor in human fibroblasts. *Proc. Natl. Acad. Sci. USA* 80, 5592-5596.
23. Waterfield, M.D., Scrace, G.T., Whittle, N., Stroobant, P., Johnsson, A., Wasteson, Å., Westermark, B., Heldin, C.-H., Huang, J.S., and Deuel, T.F. (1983). Platelet-derived growth factor is structurally related to the putative transforming protein p28^{sis} of simian sarcoma virus. *Nature* 304, 35-39.
24. Betsholtz, C., Heldin, C.-H., Nistér, M., Ek, B., Wasteson, Å., and Westermark, B. (1983). Synthesis of a PDGF-like growth factor in human glioma and sarcoma cells suggests the expression of the cellular homologue to the transforming protein of simian sarcoma virus. *Biochem. Biophys. Res. Commun.* 117, 176-182.
25. Nistér, M., Heldin, C.-H., Wasteson, Å., and Westermark, B. (1984). A glioma-derived analog to platelet-derived growth factor: Demonstration of receptor competing activity and immunological crossreactivity. *Proc. Natl. Acad. Sci. USA* 81, 926-930.
26. Ek, B., and Heldin, C.-H. (1984). Use of an antiserum against phosphotyrosine for the identification of phosphorylated components in human fibroblasts stimulated by platelet-derived growth factor. *J. Biol. Chem.* 259, 11145-11152.
27. Johnsson, A., Heldin, C.-H., Wasteson, Å., Westermark, B., Deuel, T.F., Huang, J.S., Seeburg, P.H., Gray, A., Ullrich, A., Scrace, G., Stroobant, P., and Waterfield, M.D. (1984). The *c-sis* gene encodes a precursor of the B chain of platelet-derived growth factor. *EMBO J.* 3, 921-928.
28. Betsholtz, C., Westermark, B., Ek, B., and Heldin, C.-H. (1984). Coexpression of a PDGF-like growth factor and PDGF receptors in a human osteosarcoma cell line: Implications for autocrine receptor activation. *Cell* 39, 447-457.

29. Johnsson, A., Betsholtz, C., von der Helm, K., Heldin, C.-H., and Westermark, B. (1985). Platelet-derived growth factor agonist activity of a secreted form of the *v-sis* oncogene product. *Proc. Natl. Acad. Sci. USA* 82, 1721-1725.
30. Westermark, B., and Heldin, C.-H. (1985). Similar action of platelet-derived growth factor and epidermal growth factor in the prereplicative phase of human fibroblasts suggests a common intracellular pathway. *J. Cell. Physiol.* 124, 43-48.
31. Goustin, A.S., Betsholtz, C., Pfeifer-Ohlsson, S., Persson, H., Rydnert, J., Bywater, M., Holmgren, G., Heldin, C.-H., Westermark, B., and Ohlsson, R. (1985). Coexpression of the *sis* and *myc* proto-oncogenes in developing human placenta suggests autocrine control of trophoblast growth. *Cell* 41, 301-312.
32. Betsholtz, C., Bywater, M., Westermark, B., Burk, R.R., and Heldin, C.-H. (1985). Expression of the *c-sis* gene and secretion of a platelet-derived growth factor-like protein by simian virus 40-transformed BHK cells. *Biochem. Biophys. Res. Commun.* 130, 753-760.
33. Nilsson, J., Sjölund, M., Palmberg, L., Thyberg, J., and Heldin, C.-H. (1985). Arterial smooth muscle cells in primary culture produce a platelet-derived growth factor-like protein. *Proc. Natl. Acad. Sci. USA* 82, 4418-4422.
34. Bauer, G., Birnbaum, U., Höfler, P., and Heldin, C.-H. (1985). EBV-inducing factor from platelets exhibits growth-promoting activity for NIH 3T3 cells. *EMBO J.* 4, 1957-1961.
35. van Zoelen, E.J.J., van de Ven, W.J.M., Franssen, H.J., van Oostwaard, T.M.J., van der Saag, P.T., Heldin, C.-H., and de Laat, S.W. (1985). Neuroblastoma cells express *c-sis* and produce a transforming growth factor antigenically related to the platelet-derived growth factor. *Mol. Cell. Biol.* 5, 2289-2297.
36. Johnsson, A., Betsholtz, C., Heldin, C.-H., and Westermark, B. (1985). Antibodies against platelet-derived growth factor inhibit acute transformation by simian sarcoma virus. *Nature* 317, 438-440.
37. Nistér, M., Heldin, C.-H., and Westermark, B. (1986). Clonal variation in the production of a platelet-derived growth factor-like protein and expression of corresponding receptors in a human malignant glioma. *Cancer Research* 46, 332-340.
38. Ek, B., and Heldin, C.-H. (1986). Specific cleavage of the fibroblast receptor for platelet-derived growth factor by an endogenous Ca^{2+} -dependent thiol protease. *Eur. J. Biochem.* 155, 409-413.
39. Heldin, C.-H., Johnsson, A., Wennergren, S., Wernstedt, C., Betsholtz, C., and Westermark, B. (1986). A human osteosarcoma cell line secretes a growth factor structurally related to a homodimer of PDGF A-chains. *Nature* 319, 511-514.
40. Betsholtz, C., Johnsson, A., Heldin, C.-H., and Westermark, B. (1986). Efficient reversion of simian sarcoma virus-transformation and inhibition of growth factor-induced mitogenesis by suramin. *Proc. Natl. Acad. Sci. USA* 83, 6440-6444.
41. Betsholtz, C., Johnsson, A., Heldin, C.-H., Westermark, B., Lind, P., Urdea, M.S., Eddy, R., Shows, T.B., Philpott, K., Mellor, A.L., Knott, T.J., and Scott, J. (1986). cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumour cell lines. *Nature* 320, 695-699.
42. Johnsson, A., Betsholtz, C., Heldin, C.-H., and Westermark, B. (1986). The phenotypic characteristics of simian sarcoma virus-transformed human fibroblasts suggest that the *v-sis* gene product acts solely as a PDGF receptor agonist in cell transformation. *EMBO J.* 5, 1535-1541.

43. Westermark, B., Johnsson, A., Paulsson, Y., Betsholtz, C., Heldin, C.-H., Herlyn, M., Rodeck, U., and Koprowski, H. (1986). Human melanoma cell lines of primary and metastatic origin express the genes encoding the chains of platelet-derived growth factor (PDGF) and produce a PDGF-like growth factor. *Proc. Natl. Acad. Sci. USA* 83, 7197-7200.
44. Sejersen, T., Betsholtz, C., Sjölund, M., Heldin, C.-H., Westermark, B., and Thyberg, J. (1986). Rat skeletal myoblasts and arterial smooth muscle cells express the gene for the A chain but not the gene for the B chain (*c-sis*) of platelet-derived growth factor (PDGF) and produce a PDGF-like protein. *Proc. Natl. Acad. Sci. USA* 83, 6844-6848.
45. Paulsson, Y., Bywater, M., Pfeifer-Ohlsson, S., Ohlsson, R., Nilsson, S., Heldin, C.-H., Westermark, B., and Betsholtz, C. (1986). Growth factors induce early pre-replicative changes in senescent human fibroblasts. *EMBO J.* 5, 2157-2162.
46. Cooper, C.S., Tempest, P.R., Beckman, M.P., Heldin, C.-H., and Brookes, P. (1986). Amplification and overexpression of the *met* gene in spontaneously transformed NIH3T3 mouse fibroblasts. *EMBO J.* 5, 2623-2628.
47. Betsholtz, C., Bergh, J., Bywater, M., Pettersson, M., Johnsson, A., Heldin, C.-H., Ohlsson, R., Knott, T.J., Scott, J., Bell, G.I., and Westermark, B. (1987). Expression of multiple growth factors in a human lung cancer cell line. *Int. J. Cancer* 39, 502-507.
48. Rönstrand, L., Beckmann, M.P., Faulders, B., Östman, A., Ek, B., and Heldin, C.-H. (1987). Purification of the receptor for platelet-derived growth factor from porcine uterus. *J. Biol. Chem.* 262, 2929-2932.
49. Collins, T., Pober, J.S., Gimbrone, M.A.J., Hammacher, A., Betsholtz, C., Westermark, B., and Heldin, C.-H. (1987). Cultured human endothelial cells express platelet-derived growth factor A chain. *Am. J. Pathol.* 126, 7-12.
50. Paulsson, Y., Bywater, M., Heldin, C.-H., and Westermark, B. (1987). Effects of epidermal growth factor and platelet-derived growth factor on *c-fos* and *c-myc* mRNA levels in normal human fibroblasts. *Exp. Cell Res.* 171, 186-194.
51. Peres, R., Betsholtz, C., Westermark, B., and Heldin, C.-H. (1987). Frequent expression of growth factors for mesenchymal cells in human mammary carcinoma cell lines. *Cancer Res.* 47, 3425-3429.
52. Miyazono, K., Okabe, T., Urabe, A., Takaku, F., and Heldin, C.-H. (1987). Purification and properties of an endothelial cell growth factor from human platelets. *J. Biol. Chem.* 262, 4098-4103.
53. Paulsson, Y., Hammacher, A., Heldin, C.-H., and Westermark, B. (1987). Possible positive autocrine feedback in the prereplicative phase of human fibroblasts. *Nature* 328, 715-717.
54. Böhmer, F.-D., Kraft, R., Otto, A., Wernstedt, C., Hellman, U., Kurtz, A., Müller, T., Rohde, K., Eitzold, G., Lehmann, W., Langen, P., Heldin, C.-H., and Grosse, R. (1987). Identification of a polypeptide growth inhibitor from bovine mammary gland. *J. Biol. Chem.* 262, 15137-15143.
55. Alitalo, R., Andersson, L.C., Betsholtz, C., Nilsson, K., Westermark, B., Heldin, C.-H., and Alitalo, K. (1987). Induction of platelet-derived growth factor gene expression during megakaryoblastic and monocytic differentiation of human leukemia cell lines. *EMBO J.* 6, 1213-1218.
56. Mäkelä, T.P., Alitalo, R., Paulsson, Y., Westermark, B., Heldin, C.-H., and Alitalo, K. (1987). Regulation of platelet-derived growth factor gene expression by transforming growth factor β and phorbol ester in human leukemia cell lines. *Mol. Cell. Biol.* 7, 3656-3662.

57. Claesson-Welsh, L., Rönnstrand, L., and Heldin, C.-H. (1987). Biosynthesis and intracellular transport of the receptor for platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA* 84, 8796-8800.
58. Frampton, G., Hildreth, G., Hartley, B., Cameron, J.C., Heldin, C.-H., and Wasteson, A. (1988). Could platelet-derived growth factor have a role in the pathogenesis of lupus nephritis? *Lancet* ii, 343.
59. Rubin, K., Terracio, L., Rönnstrand, L., Heldin, C.-H., and Klareskog, L. (1988). Expression of platelet-derived growth factor receptors is induced on connective tissue cells during chronic synovial inflammation. *Scand. J. Immunol.* 27, 285-294.
60. Rönnstrand, L., Terracio, L., Claesson-Welsh, L., Heldin, C.-H., and Rubin, K. (1988). Characterization of two monoclonal antibodies reactive with the external domain of the platelet-derived growth factor receptor. *J. Biol. Chem.* 263, 10429-10435.
61. Sjölund, M., Hedin, U., Sejersen, T., Heldin, C.-H., and Thyberg, J. (1988). Arterial smooth muscle cells express platelet-derived growth factor (PDGF) A chain mRNA, secrete a PDGF-like mitogen, and bind exogenous PDGF in a phenotype- and growth state-dependent manner. *J. Cell Biol.* 106, 403-413.
62. Nistér, M., Libermann, T.A., Betsholtz, C., Pettersson, M., Claesson-Welsh, L., Heldin, C.-H., Schlessinger, J., and Westermark, B. (1988). Expression of messenger RNAs for platelet-derived growth factor and transforming growth factor- α and their receptors in human malignant glioma cell lines. *Cancer Res.* 48, 3910-3918.
63. Beckmann, M.P., Betsholtz, C., Heldin, C.-H., Westermark, B., Di Marco, E., Di Fiore, P.P., Robbins, K.C., and Aaronson, S.A. (1988). Comparison of biological properties and transforming potential of human PDGF-A and PDGF-B chains. *Science* 241, 1346-1349.
64. Swenne, I., Heldin, C.-H., Hill, D.J., and Hellerström, C. (1988). Effects of platelet-derived growth factor and somatomedin-C/insulin-like growth factor I on the deoxyribonucleic acid replication of fetal rat islets of Langerhans in tissue culture. *Endocrinology* 122, 214-218.
65. Hammacher, A., Hellman, U., Johnsson, A., Östman, A., Gunnarsson, K., Westermark, B., Wasteson, A., and Heldin, C.-H. (1988). A major part of platelet-derived growth factor purified from human platelets is a heterodimer of one A and one B chain. *J. Biol. Chem.* 263, 16493-16498.
66. Hammacher, A., Nistér, M., Westermark, B., and Heldin, C.-H. (1988). A human glioma cell line secretes three structurally and functionally different dimeric forms of platelet-derived growth factor. *Eur. J. Biochem.* 176, 179-186.
67. Nistér, M., Hammacher, A., Mellström, K., Siegbahn, A., Rönnstrand, L., Westermark, B., and Heldin, C.-H. (1988). A glioma-derived PDGF A chain homodimer has different functional activities from a PDGF AB heterodimer purified from human platelets. *Cell* 52, 791-799.
68. Miyazono, K., Hellman, U., Wernstedt, C., and Heldin, C.-H. (1988). Latent high molecular weight complex of transforming growth factor β 1. Purification from human platelets and structural characterization. *J. Biol. Chem.* 263, 6407-6415.
69. Mellström, K., Heldin, C.-H., and Westermark, B. (1988). Induction of circular membrane ruffling on human fibroblasts by platelet-derived growth factor. *Exp. Cell Res.* 177, 347-359.
70. Östman, A., Rall, L., Hammacher, A., Wormstead, M.A., Coit, D., Valenzuela, P., Betsholtz, C., Westermark, B., and Heldin, C.-H. (1988). Synthesis and assembly of a functionally active recombinant platelet-derived growth factor AB heterodimer. *J. Biol. Chem.* 263, 16202-16208.

71. Claesson-Welsh, L., Eriksson, A., Morén, A., Severinsson, L., Ek, B., Östman, A., Betsholtz, C., and Heldin, C.-H. (1988). cDNA cloning and expression of a human platelet-derived growth factor (PDGF) receptor specific for B-chain-containing PDGF molecules. *Mol. Cell. Biol.* 8, 3476-3486.
72. Bywater, M., Rorsman, F., Bongcam-Rudloff, E., Mark, G., Hammacher, A., Heldin, C.-H., Westermark, B., and Betsholtz, C. (1988). Expression of recombinant platelet-derived growth factor A- and B-chain homodimers in Rat-1 cells and human fibroblasts reveals differences in protein processing and autocrine effects. *Mol. Cell. Biol.* 8, 2753-2762.
73. Hermansson, M., Nistér, M., Betsholtz, C., Heldin, C.-H., Westermark, B., and Funai, K. (1988). Endothelial cell hyperplasia in human glioblastoma: Coexpression of mRNA for platelet-derived growth factor (PDGF) B chain and PDGF receptor suggests autocrine growth stimulation. *Proc. Natl. Acad. Sci. USA* 85, 7748-7752.
74. Heldin, C.-H., Bäckström, G., Östman, A., Hammacher, A., Rönstrand, L., Rubin, K., Nistér, M., and Westermark, B. (1988). Binding of different dimeric forms of PDGF to human fibroblasts: evidence for two separate receptor types. *EMBO J.* 7, 1387-1393.
75. Rubin, K., Tingström, A., Hansson, G.K., Larsson, E., Rönstrand, L., Klareskog, L., Claesson-Welsh, L., Heldin, C.-H., Fellström, B., and Terracio, L. (1988). Induction of B-type receptors for platelet-derived growth factor in vascular inflammation: Possible implications for development of vascular proliferative lesions. *Lancet* i, 1353-1356.
76. Terracio, L., Rönstrand, L., Tingström, A., Rubin, K., Claesson-Welsh, L., Funai, K., and Heldin, C.-H. (1988). Induction of platelet-derived growth factor receptor expression in smooth muscle cells and fibroblasts upon tissue culturing. *J. Cell Biol.* 107, 1947-1957.
77. Paulsson, Y., Beckmann, M.P., Westermark, B., and Heldin, C.-H. (1988). Density-dependent inhibition of cell growth by transforming growth factor- β 1 in normal human fibroblasts. *Growth Factors* 1, 19-27.
78. Heldin, N.-E., Gustavsson, B., Claesson-Welsh, L., Hammacher, A., Mark, J., Heldin, C.-H., and Westermark, B. (1988). Aberrant expression of receptors for platelet-derived growth factor in an anaplastic thyroid carcinoma cell line. *Proc. Natl. Acad. Sci. USA* 85, 9302-9306.
79. Paulsson, Y., Austgulen, R., Hofslø, E., Heldin, C.-H., Westermark, B., and Nissen-Meyer, J. (1989). Tumor necrosis factor-induced expression of platelet-derived growth factor A-chain messenger RNA in fibroblasts. *Exp. Cell Res.* 180, 490-496.
80. Miyazono, K., and Heldin, C.-H. (1989). High-yield purification of platelet-derived endothelial cell growth factor: Structural characterization and establishment of a specific antiserum. *Biochemistry* 28, 1704-1710.
81. Heldin, N.-E., Paulsson, Y., Forsberg, K., Heldin, C.-H., and Westermark, B. (1989). Induction of cyclic AMP synthesis by forskolin is followed by a reduction in the expression of c-myc messenger RNA and inhibition of ^3H -thymidine incorporation in human fibroblasts. *J. Cell. Phys.* 138, 17-23.
82. Severinsson, L., Claesson-Welsh, L., and Heldin, C.-H. (1989). A B-type PDGF receptor lacking most of the intracellular domain escapes degradation after ligand binding. *Eur. J. Biochem.* 182, 679-686.
83. Claesson-Welsh, L., Hammacher, A., Westermark, B., Heldin, C.-H., and Nistér, M. (1989). Identification and structural analysis of the A type receptor for platelet-derived growth factor. Similarities with the B type receptor. *J. Biol. Chem.* 264, 1742-1747.

84. Claesson-Welsh, L., Eriksson, A., Westermark, B., and Heldin, C.-H. (1989). cDNA cloning and expression of the human A-type platelet-derived growth factor (PDGF) receptor establishes structural similarity to the B-type PDGF receptor. *Proc. Natl. Acad. Sci. USA* 86, 4917-4921.
85. Östman, A., Bäckström, G., Fong, N., Betsholtz, C., Wernstedt, C., Hellman, U., Westermark, B., Valenzuela, P., and Heldin, C.-H. (1989). Expression of three recombinant homodimeric isoforms of PDGF in *Saccharomyces cerevisiae*: Evidence for difference in receptor binding and functional activities. *Growth Factors* 1, 271-281.
86. Fellström, B., Klareskog, L., Heldin, C.-H., Larsson, E., Rönstrand, L., Terracio, L., Tufveson, G., Wahlberg, J., and Rubin, K. (1989). Platelet-derived growth factor receptors in the kidney - Upregulated expression in inflammation. *Kidney Int.* 36, 1099-1102.
87. Heldin, C.-H., Emlund, A., Rorsman, C., and Rönstrand, L. (1989). Dimerization of B-type platelet-derived growth factor receptors occurs after ligand binding and is closely associated with receptor kinase activation. *J. Biol. Chem.* 264, 8905-8912.
88. Heldin, P., Laurent, T.C., and Heldin, C.-H. (1989). Effect of growth factors on hyaluronan synthesis in cultured human fibroblasts. *Biochem. J.* 258, 919-922.
89. Miyazono, K., and Heldin, C.-H. (1989). Role for carbohydrate structures in TGF- β 1 latency. *Nature* 338, 158-160.
90. Kovacina, K.S., Steele-Perkins, G., Purchio, A.F., Lioubin, M., Miyazono, K., Heldin, C.-H., and Roth, R.A. (1989). Interactions of recombinant and platelet transforming growth factor- β 1 precursor with the insulin-like growth factor II/mannose 6-phosphate receptor. *Biochem. Biophys. Res. Commun.* 160, 393-403.
91. Ishikawa, F., Miyazono, K., Hellman, U., Drexler, H., Wernstedt, C., Hagiwara, K., Usuki, K., Takaku, F., Risau, W., and Heldin, C.-H. (1989). Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. *Nature* 338, 557-562.
92. Hart, I.K., Richardson, W.D., Heldin, C.-H., Westermark, B., and Raff, M.C. (1989). PDGF receptors on cells of the oligodendrocyte-type-2 astrocyte (O-2A) cell lineage. *Development* 105, 595-603.
93. Pringle, N., Collarini, E.J., Mosley, M.J., Heldin, C.-H., Westermark, B., and Richardson, W.D. (1989). PDGF A chain homodimers drive proliferation of bipotential (O-2A) glial progenitor cells in the developing rat optic nerve. *EMBO J.* 8, 1049-1056.
94. Usuki, K., Heldin, N.-E., Miyazono, K., Ishikawa, F., Takaku, F., Westermark, B., and Heldin, C.-H. (1989). Production of platelet-derived endothelial cell growth factor by normal and transformed human cells in culture. *Proc. Natl. Acad. Sci. USA* 86, 7427-7431.
95. Hammacher, A., Nistér, M., and Heldin, C.-H. (1989). The A-type receptor for platelet-derived growth factor mediates protein tyrosine phosphorylation, receptor transmodulation and a mitogenic response. *Biochem. J.* 264, 15-20.
96. Smits, A., Hermansson, M., Nistér, M., Karnushina, I., Heldin, C.-H., Westermark, B., and Funa, K. (1989). Rat brain capillary endothelial cells express functional PDGF B-type receptors. *Growth Factors* 2, 1-8.
97. Hammacher, A., Mellström, K., Heldin, C.-H., and Westermark, B. (1989). Isoform-specific induction of actin reorganization by platelet-derived growth factor suggests that the functionally active receptor is a dimer. *EMBO J.* 8, 2489-2495.

98. Severinsson, L., Ek, B., Mellström, K., Claesson-Welsh, L., and Heldin, C.-H. (1990). Deletion of the kinase insert sequence of the platelet-derived growth factor β -receptor affects receptor kinase activity and signal transduction. *Mol. Cell. Biol.* 10, 801-809.
99. Siegbahn, A., Hammacher, A., Westermark, B., and Heldin, C.-H. (1990). Differential effects of the various isoforms of platelet-derived growth factor on chemotaxis of fibroblasts, monocytes, and granulocytes. *J. Clin. Invest.* 85, 916-920.
100. Westermark, B., Siegbahn, A., Heldin, C.-H., and Claesson-Welsh, L. (1990). B-type receptor for platelet-derived growth factor mediates a chemotactic response by means of ligand-induced activation of the receptor protein-tyrosine kinase. *Proc. Natl. Acad. Sci. USA* 87, 128-132.
101. Funa, K., Papanicolaou, V., Juhlin, C., Rastad, J., Åkerström, G., Heldin, C.-H., and Öberg, K. (1990). Expression of platelet-derived growth factor β -receptors on stromal tissue cells in human carcinoid tumors. *Cancer Res.* 50, 748-753.
102. Rönstrand, L., Sorokin, A., Engström, U., and Heldin, C.-H. (1990). Characterization of the platelet-derived growth factor β -receptor kinase activity by use of synthetic peptides. *Biochem. Biophys. Res. Commun.* 167, 1333-1340.
103. Werner, S., Hofschneider, P.H., Heldin, C.-H., Östman, A., and Roth, W.K. (1990). Cultured Kaposi's sarcoma-derived cells express functional PDGF A-type and B-type receptors. *Exp. Cell Res.* 187, 98-103.
104. Welsh, M., Claesson-Welsh, L., Hallberg, A., Welsh, N., Betsholtz, C., Arkhammar, P., Nilsson, T., Heldin, C.-H., and Berggren, P.-O. (1990). Coexpression of the platelet-derived growth factor (PDGF) B chain and the PDGF β receptor in isolated pancreatic islet cells stimulates DNA synthesis. *Proc. Natl. Acad. Sci. USA* 87, 5807-5811.
105. Jin, P., Rahm, M., Claesson-Welsh, L., Heldin, C.-H., and Sejersen, T. (1990). Expression of PDGF A-chain and β -receptor genes during rat myoblast differentiation. *J. Cell Biol.* 110, 1665-1672.
106. Kurtz, A., Vogel, F., Funa, K., Heldin, C.-H., and Grosse, R. (1990). Developmental regulation of mammary-derived growth inhibitor expression in bovine mammary tissue. *J. Cell Biology* 110, 1779-1789.
107. Leveen, P., Claesson-Welsh, L., Heldin, C.-H., Westermark, B., and Betsholtz, C. (1990). Expression of messenger RNAs for platelet-derived growth factor and its receptors in human sarcoma cell lines. *Int. J. Cancer* 46, 1066-1070.
108. Sjölund, M., Rahm, M., Claesson-Welsh, L., Sejersen, T., Heldin, C.-H., and Thyberg, J. (1990). Expression of PDGF α - and β -receptors in rat arterial smooth muscle cells is phenotype and growth state dependent. *Growth Factors* 3, 191-203.
109. Usuki, K., Norberg, L., Larsson, E., Miyazono, K., Hellman, U., Wernstedt, C., Rubin, K., and Heldin, C.-H. (1990). Localization of platelet-derived endothelial cell growth factor in human placenta and purification of an alternatively processed form. *Cell Regulation* 1, 577-584.
110. Kanzaki, T., Olofsson, A., Morén, A., Wernstedt, C., Hellman, U., Miyazono, K., Claesson-Welsh, L., and Heldin, C.-H. (1990). TGF- β 1 binding protein: A component of the large latent complex of TGF- β 1 with multiple repeat sequences. *Cell* 61, 1051-1061.
111. Thyberg, J., Östman, A., Bäckström, G., Westermark, B., and Heldin, C.-H. (1990). Localization of platelet-derived growth factor (PDGF) in CHO cells transfected with PDGF A- or B-chain cDNA: retention of PDGF-BB in the endoplasmic reticulum and Golgi complex. *J. Cell Science* 97, 219-229.

112. Sorkin, A., Westermarck, B., Heldin, C.-H., and Claesson-Welsh, L. (1991). Effect of receptor kinase inactivation on the rate of internalization and degradation of PDGF and the PDGF β -receptor. *J. Cell Biol.* 112, 469-478.
113. Östman, A., Andersson, M., Betsholtz, C., Westermarck, B., and Heldin, C.-H. (1991). Identification of a cell retention signal in the B-chain of platelet-derived growth factor and in the long splice version of the A-chain. *Cell Regul.* 2, 503-512.
114. Reuter Dahl, C., Tingström, A., Terracio, L., Funa, K., Heldin, C.-H., and Rubin, K. (1991). Characterization of platelet-derived growth factor β -receptor expressing cells in the vasculature of human rheumatoid synovium. *Lab. Invest.* 64, 321-329.
115. Östman, A., Andersson, M., Hellman, U., and Heldin, C.-H. (1991). Identification of three amino acids in the platelet-derived growth factor (PDGF) B-chain that are important for binding to the PDGF β -receptor. *J. Biol. Chem.* 266, 10073-10077.
116. Miyazono, K., Olofsson, A., Colosetti, P., and Heldin, C.-H. (1991). A role of the latent TGF- β 1-binding protein in the assembly and secretion of TGF- β 1. *EMBO J.* 10, 1091-1101.
117. Smits, A., Kato, M., Westermarck, B., Nistér, M., Heldin, C.-H., and Funa, K. (1991). Neurotrophic activity of platelet-derived growth factor (PDGF): Rat neuronal cells possess functional PDGF β -type receptors and respond to PDGF. *Proc. Natl. Acad. Sci. USA* 88, 8159-8163.
118. Ichijo, H., Rönnstrand, L., Miyagawa, K., Ohashi, H., Heldin, C.-H., and Miyazono, K. (1991). Purification of transforming growth factor- β 1 binding proteins from porcine uterus membranes. *J. Biol. Chem.* 266, 22459-22464.
119. Hagiwara, K., Stenman, G., Honda, H., Sahlin, P., Andersson, A., Miyazono, K., Heldin, C.-H., Ishikawa, F., and Takaku, F. (1991). Organization and chromosomal localization of the human platelet-derived endothelial cell growth factor gene. *Mol. Cell. Biol.* 11, 2125-2132.
120. Heldin, P., Pertoft, H., Nordlinder, H., Heldin, C.-H., and Laurent, T.C. (1991). Differential expression of platelet-derived growth factor α - and β -receptors on fat-storing cells and endothelial cells of rat liver. *Exp. Cell Res.* 193, 364-369.
121. Usuki, K., Miyazono, K., and Heldin, C.-H. (1991). Covalent linkage between nucleotides and platelet-derived endothelial cell growth factor. *J. Biol. Chem.* 266, 20525-20531.
122. Blume-Jensen, P., Claesson-Welsh, L., Siegbahn, A., Zsebo, K.M., Westermarck, B., and Heldin, C.-H. (1991). Activation of the human *c-kit* product by ligand-induced dimerization mediates circular actin reorganization and chemotaxis. *EMBO J.* 10, 4121-4128.
123. Nistér, M., Claesson-Welsh, L., Eriksson, A., Heldin, C.-H., and Westermarck, B. (1991). Differential expression of platelet-derived growth factor receptors in human malignant glioma cell lines. *J. Biol. Chem.* 266, 16755-16763.
124. Eriksson, A., Nistér, M., Leveen, P., Westermarck, B., Heldin, C.-H., and Claesson-Welsh, L. (1991). Induction of platelet-derived growth factor α - and β -receptor mRNA and protein by platelet-derived growth factor BB. *J. Biol. Chem.* 266, 21136-21144.
125. Versnel, M.A., Claesson-Welsh, L., Hammacher, A., Bouts, M.J., van der Kwast, T.H., Eriksson, A., Willemsen, R., Weima, S.M., Hoogsteden, H.C., Hagemeijer, A., and Heldin, C.-H. (1991). Human malignant mesothelioma cell lines express PDGF β -receptors whereas cultured normal mesothelial cells express predominantly PDGF α -receptors. *Oncogene* 6, 2005-2011.

126. Mori, S., Claesson-Welsh, L., and Heldin, C.-H. (1991). Identification of a hydrophobic region in the carboxyl terminus of the platelet-derived growth factor β -receptor which is important for ligand-mediated endocytosis. *J. Biol. Chem.* 266, 21158-21164.
127. Eriksson, A., Rorsman, C., Ernlund, A., Claesson-Welsh, L., and Heldin, C.-H. (1992). Ligand-induced homo- and hetero-dimerization of platelet-derived growth factor α - and β -receptors in intact cells. *Growth Factors* 6, 1-14.
128. Stenman, G., Sahlin, P., Dumanski, J.P., Hagiwara, K., Ishikawa, F., Miyazono, K., Collins, V.P., and Heldin, C.-H. (1992). Regional localization of the human platelet-derived endothelial cell growth factor (ECGF1) gene to chromosome 22q13. *Cytogenet. Cell Genet.* 59, 22-23.
129. Lepistö, J., Laato, M., Niinikoski, J., Lundberg, C., Gerdin, B., and Heldin, C.-H. (1992). Effects of homodimeric isoforms of platelet-derived growth factor (PDGF-AA and PDGF-BB) on wound healing in rat. *J. Surgical Research* 53, 596-601.
130. Östman, A., Thyberg, J., Westermarck, B., and Heldin, C.-H. (1992). PDGF-AA and PDGF-BB biosynthesis: Proprotein processing in the Golgi complex and lysosomal degradation of PDGF-BB retained intracellularly. *J. Cell. Biol.* 118, 509-519.
131. Smits, A., Funa, K., Vassbotn, F.S., Beausang-Linder, M., af Ekenstam, F., Heldin, C.-H., Westermarck, B., and Nistér, M. (1992). Expression of platelet-derived growth factor and its receptors in proliferative disorders of fibroblastic origin. *Am. J. Pathol.* 140, 639-648.
132. Vassbotn, F.S., Östman, A., Siegbahn, A., Holmsen, H., and Heldin, C.-H. (1992). Neomycin is a platelet-derived growth factor (PDGF) antagonist that allows discrimination of PDGF α - and β -receptor signals in cells expressing both receptor types. *J. Biol. Chem.* 267, 15635-15641.
133. Tingström, A., Heldin, C.-H., and Rubin, K. (1992). Regulation of fibroblast-mediated collagen gel contraction by platelet-derived growth factor, interleukin-1 α and transforming growth factor- β 1. *J. Cell Sci.* 102, 315-322.
134. Tingström, A., Reuterdaahl, C., Lindahl, P., Heldin, C.-H., and Rubin, K. (1992). Expression of platelet-derived growth factor- β receptors on human fibroblasts. Regulation by recombinant platelet-derived growth factor-BB, IL-1, and tumor necrosis factor- α . *J. Immunol.* 148, 546-554.
135. Holmgren, L., Claesson-Welsh, L., Heldin, C.-H., and Ohlsson, R. (1992). The expression of PDGF α - and β -receptors in subpopulations of PDGF-producing cells implicates autocrine stimulatory loops in the control of proliferation in cytrophoblasts that have invaded the maternal endometrium. *Growth Factors* 6, 219-231.
136. Chaudhry, A., Papanicolaou, V., Öberg, K., Heldin, C.-H., and Funa, K. (1992). Platelet-derived growth factors and their receptors in neuroendocrine tumors of the digestive system. *Cancer Res.* 52, 1006-1012.
137. Miyazono, K., Thyberg, J., and Heldin, C.-H. (1992). Retention of the transforming growth factor- β 1 precursor in the Golgi complex in a latent endoglycosidase H-sensitive form. *J. Biol. Chem.* 267, 5668-5675.
138. Versnel, M.A., Bouts, M.J., Langerak, A.W., van der Kwast, T.H., Hoogsteden, H.C., Hagemijer, A., and Heldin, C.-H. (1992). Hydrocortisone-induced increase of PDGF β -receptor expression in a human malignant mesothelioma cell line. *Exp. Cell Res.* 200, 83-88.
139. Eriksson, A., Siegbahn, A., Westermarck, B., Heldin, C.-H., and Claesson-Welsh, L. (1992). PDGF α - and β -receptors activate unique and common signal transduction pathways. *EMBO J.* 11, 543-550.

140. Rönstrand, L., Mori, S., Arvidsson, A.-K., Eriksson, A., Wernstedt, C., Hellman, U., Claesson-Welsh, L., and Heldin, C.-H. (1992). Identification of two C-terminal autophosphorylation sites in the PDGF β -receptor: involvement in the interaction with phospholipase C- γ . *EMBO J.* 11, 3911-3919.
141. Mori, S., Heldin, C.-H., and Claesson-Welsh, L. (1992). Ligand-induced polyubiquitination of the platelet-derived growth factor β -receptor. *J. Biol. Chem.* 267, 6429-6434.
142. Waltenberger, J., Usuki, K., Fellström, B., Funa, K., and Heldin, C.-H. (1992). Platelet-derived endothelial cell growth factor: Pharmacokinetics, organ distribution and degradation after intravenous administration in rats. *FEBS Lett.* 313, 129-132.
143. Olofsson, A., Miyazono, K., Kanzaki, T., Colosetti, P., Engström, U., and Heldin, C.-H. (1992). Transforming growth factor- β 1, - β 2 and - β 3 secreted by a human glioblastoma cell line: Identification of small and different forms of large latent complexes. *J. Biol. Chem.* 267, 19482-19488.
144. Hermansson, M., Funa, K., Hartman, M., Claesson-Welsh, L., Heldin, C.-H., Westermarck, B., and Nistér, M. (1992). Platelet-derived growth factor and its receptors in human glioma tissue: Expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. *Cancer Res.* 52, 3213-3219.
145. Engström, U., Engström, Å., Ernlund, A., Westermarck, B., and Heldin, C.-H. (1992). Identification of a peptide antagonist for platelet-derived growth factor. *J. Biol. Chem.* 267, 16581-16587.
146. Risau, W., Drexler, H., Mironov, V., Smits, A., Siegbahn, A., Funa, K., and Heldin, C.-H. (1992). Platelet-derived growth factor is angiogenic *in vivo*. *Growth Factors* 7, 261-266.
147. Andersson, M., Östman, A., Bäckström, G., Hellman, U., George-Nascimento, C., Westermarck, B., and Heldin, C.-H. (1992). Assignment of interchain disulfide bonds in platelet-derived growth factor (PDGF) and evidence for agonist activity of monomeric PDGF. *J. Biol. Chem.* 267, 11260-11266.
148. Usuki, K., Saras, J., Waltenberger, J., Miyazono, K., Pierce, G., Thomason, A., and Heldin, C.-H. (1992). Platelet-derived endothelial cell growth factor has thymidine phosphorylase activity. *Biochem. Biophys. Res. Commun.* 184, 1311-1316.
149. Arvidsson, A.-K., Heldin, C.-H., and Claesson-Welsh, L. (1992). Transduction of circular membrane ruffling by the platelet-derived growth factor β -receptor is dependent on its kinase insert. *Cell Growth & Diff.* 3, 881-887.
150. Mori, S., Heldin, C.-H., and Claesson-Welsh, L. (1993). Ligand-induced ubiquitination of the platelet-derived growth factor β -receptor plays a negative regulatory role in its mitogenic signaling. *J. Biol. Chem.* 268, 577-583.
151. Forsberg, K., Valyi-Nagy, I., Heldin, C.-H., Herlyn, M., and Westermarck, B. (1993). Platelet-derived growth factor (PDGF) in oncogenesis: Development of a vascular connective tissue stroma in xenotransplanted human melanoma producing PDGF-BB. *Proc. Natl. Acad. Sci. USA* 90, 393-397.
152. Waltenberger, J., Lundin, L., Öberg, K., Willander, E., Miyazono, K., Heldin, C.-H., and Funa, K. (1993). Involvement of transforming growth factor- β in the formation of fibrotic lesions in carcinoid heart disease. *Am. J. Pathol.* 142, 71-78.
153. ten Dijke, P., Ichijo, H., Franzén, P., Schulz, P., Saras, J., Toyoshima, H., Heldin, C.-H., and Miyazono, K. (1993). Activin receptor-like kinases: a novel subclass of cell-surface receptors with predicted serine/threonine kinase activity. *Oncogene* 8, 2879-2887.

154. Franzén, P., ten Dijke, P., Ichijo, H., Yamashita, H., Schulz, P., Heldin, C.-H., and Miyazono, K. (1993). Cloning of a TGF β type I receptor that forms a heteromeric complex with the TGF β type II receptor. *Cell* 75, 681-692.
155. Sorkin, A., Eriksson, A., Heldin, C.H., Westermark, B., and Claesson-Welsh, L. (1993). Pool of ligand-bound platelet-derived growth factor beta-receptors remain activated and tyrosine phosphorylated after internalization. *J. Cell. Physiol.* 156, 373-382.
156. Colosetti, P., Hellman, U., Heldin, C.-H., and Miyazono, K. (1993). Ca²⁺ binding of latent transforming growth factor- β 1 binding protein. *FEBS Lett.* 320, 140-144.
157. Eccleston, P.A., Funa, K., and Heldin, C.-H. (1993). Expression of platelet-derived growth factor (PDGF) and PDGF α - and β -receptors in the peripheral nervous system: An analysis of sciatic nerve and dorsal root ganglia. *Dev. Biol.* 155, 459-470.
158. Vassbotn, F.S., Andersson, M., Westermark, B., Heldin, C.-H., and Östman, A. (1993). Reversion of autocrine transformation by a dominant negative platelet-derived growth factor mutant. *Mol. Cell. Biol.* 13, 4066-4076.
159. Waltenberger, J., Wanders, A., Fellström, B., Miyazono, K., Heldin, C.-H., and Funa, K. (1993). Induction of transforming growth factor- β during cardiac allograft rejection. *J. Immunol.* 151, 1147-1157.
160. Flaumenhaft, R., Abe, M., Sato, Y., Miyazono, K., Harpel, J., Heldin, C.-H., and Rifkin, D.B. (1993). Role of the latent TGF- β binding protein in the activation of latent TGF- β by co-cultures of endothelial and smooth muscle cells. *J. Cell Biol.* 120, 995-1002.
161. Mori, S., Rönstrand, L., Yokote, K., Engström, Å., Courtneidge, S.A., Claesson-Welsh, L., and Heldin, C.-H. (1993). Identification of two juxtamembrane autophosphorylation sites in the PDGF β -receptor; involvement in the interaction with Src family tyrosine kinases. *EMBO J.* 12, 2257-2264.
162. Smits, A., Odin, P., Duan, W.-M., Brundin, P., Widner, H., Heldin, C.-H., Lindwall, O., and Funa, K. (1993). Expression of platelet-derived growth factor in and around intrastriatal embryonic mesencephalic grafts. *Cell Transplantation* 2, 151-162.
163. Böhmer, F.-D., Böhmer, S.-A., and Heldin, C.-H. (1993). The dephosphorylation characteristics of the receptors for epidermal growth factor and platelet-derived growth factor in Swiss 3T3 cell membranes suggest differential regulation of receptor signalling by endogenous protein-tyrosine phosphatases. *FEBS Letters* 331, 276-280.
164. van Zoelen, E.J.J., van Rotterdam, W., van de Wetering, R.A.C., and Heldin, C.-H. (1993). Differential effects of PDGF isoforms on proliferation of normal rat kidney cells. *Growth Factors* 9, 329-339.
165. Ichijo, H., Yamashita, H., ten Dijke, P., Eto, Y., Heldin, C.-H., and Miyazono, K. (1993). Characterization of *in vivo* phosphorylation of activin type II receptor. *Biochem. Biophys. Res. Comm.* 194, 1508-1514.
166. Paulsson, Y., Karlsson, C., Heldin, C.-H., and Westermark, B. (1993). Density-dependent inhibitory effect of transforming growth factor- β 1 on human fibroblasts involves the down-regulation of platelet-derived growth factor α -receptors. *J. Cell. Physiol.* 157, 97-103.
167. Heldin, N.-E., Usuki, K., Bergh, J., Westermark, B., and Heldin, C.-H. (1993). Differential expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human lung carcinoma cell lines. *Br. J. Cancer* 68, 708-711.

168. Ichijo, H., Hellman, U., Wernstedt, C., Gonez, L.J., Claesson-Welsh, L., Heldin, C.-H., and Miyazono, K. (1993). Molecular cloning and characterization of ficolin, a multimeric protein with fibrinogen- and collagen-like domains. *J. Biol. Chem.* 268, 14505-14513.
169. Östman, A., Andersson, M., Bäckström, G., and Heldin, C.-H. (1993). Assignment of intrachain disulfide bonds in platelet-derived growth factor B-chain. *J. Biol. Chem.* 268, 13372-13377.
170. Blume-Jensen, P., Siegbahn, A., Stabel, S., Heldin, C.-H., and Rönstrand, L. (1993). Increased Kit/SCF receptor induced mitogenicity but abolished cell motility after inhibition of protein kinase C. *EMBO J.* 12, 4199-4209.
171. Pekny, M., Östman, A., Hermansson, A., Nistér, M., Heldin, C.-H., and Westermarck, B. (1994). Differences in binding to the solid substratum and extracellular matrix may explain isoform-specific paracrine effects of platelet-derived growth factor. *Growth Factors* 10, 77-87.
172. Vassbotn, F.S., Östman, A., Langeland, N., Holmsen, H., Westermarck, B., Heldin, C.-H., and Nistér, M. (1994). Activated platelet-derived growth factor autocrine pathway drives the transformed phenotype of a human glioblastoma cell line. *J. Cell. Physiol.* 158, 381-389.
173. Rupp, E., Siegbahn, A., Rönstrand, L., Wernstedt, C., Claesson-Welsh, L., and Heldin, C.-H. (1994). A unique autophosphorylation site in the PDGF α -receptor from a heterodimeric receptor complex. *Eur. J. Biochem.* 225, 29-41.
174. Wennström, S., Siegbahn, A., Yokote, K., Arvidsson, A.-K., Heldin, C.-H., Mori, S., and Claesson-Welsh, L. (1994). Membrane ruffling and chemotaxis transduced by the PDGF β -receptor require the binding site for phosphatidylinositol 3' kinase. *Oncogene* 9, 651-660.
175. Nistér, M., Enblad, P., Bäckström, G., Söderman, T., Persson, L., Heldin, C.-H., and Westermarck, B. (1994). Platelet-derived growth factor (PDGF) in neoplastic and non-neoplastic cystic lesions of the central nervous system and in cerebrospinal fluid. *Br. J. Cancer* 69, 952-956.
176. Usuki, K., Gonez, L.J., Wernstedt, C., Morén, A., Miyazono, K., Claesson-Welsh, L., and Heldin, C.-H. (1994). Structural properties of 3.0 kb and 3.2 kb transcripts encoding platelet-derived endothelial cell growth factor/thymidine phosphorylase in A431 cells. *Biochim. Biophys. Acta* 1222, 411-414.
177. Mori, S., Rönstrand, L., Claesson-Welsh, L., and Heldin, C.-H. (1994). A tyrosine residue in the juxtamembrane segment of the platelet-derived growth factor β -receptor is critical for ligand-mediated endocytosis. *J. Biol. Chem.* 269, 4917-4921.
178. Ming, X.-F., Burgering, B.M.Th., Wennström, S., Claesson-Welsh, L., Heldin, C.-H., Bos, J.L., Kozma, S.C., and Thomas, G. (1994). Activation of p70/p85 S6 kinase by a pathway independent of p21^{ras}. *Nature* 371, 426-429.
179. Andersson, M., Östman, A., Westermarck, B., and Heldin, C.-H. (1994). Characterization of the retention motif in the C-terminal part of the long splice form of platelet-derived growth factor A-chain. *J. Biol. Chem.* 269, 926-930.
180. Taketazu, F., Kato, M., Gohl, A., Ichijo, H., ten Dijke, P., Itoh, J., Kyogoku, M., Rönnelid, J., Miyazono, K., Heldin, C.-H., and Funai, K. (1994). Enhanced expression of transforming growth factor- β s and transforming growth factor- β type II receptor in the synovial tissues of patients with rheumatoid arthritis. *Lab. Invest.* 70, 620-630.
181. Taipale, J., Miyazono, K., Heldin, C.-H., and Keski-Oja, J. (1994). Latent transforming growth factor- β 1 associates to fibroblast extracellular matrix via latent TGF- β binding protein. *J. Cell Biology* 124, 171-181.

182. Chaudhry, A., Öberg, K., Gobl, A., Heldin, C.-H., and Funa, K. (1994). Expression of transforming growth factors $\beta 1$, $\beta 2$, $\beta 3$ in neuroendocrine tumors of the digestive system. *Anticancer Res.* 14, 2085-2091.
183. Blume-Jensen, P., Rönnstrand, L., Gout, I., Waterfield, M.D., and Heldin, C.-H. (1994). Modulation of Kit/stem cell factor receptor-induced signaling by protein kinase C. *J. Biol. Chem.* 269, 21793-21802.
184. Arvidsson, A.K., Rupp, E., Nånberg, E., Downward, J., Rönnstrand, L., Wennström, S., Schlessinger, J., Heldin, C.H., and Claesson-Welsh, L. (1994). Tyr-716 in the platelet-derived growth factor beta-receptor kinase insert is involved in GRB2 binding and Ras activation. *Mol. Cell. Biol.* 14, 6715-6726.
185. ten Dijke, P., Yamashita, H., Ichijo, H., Franzén, P., Laiho, M., Miyazono, K., and Heldin, C.-H. (1994). Characterization of type I receptors for transforming growth factor- β and activin. *Science* 264, 101-104.
186. Okadome, T., Yamashita, H., Franzén, P., Morén, A., Heldin, C.-H., and Miyazono, K. (1994). Distinct roles of the intracellular domains of transforming growth factor- β type I and type II receptors in signal transduction. *J. Biol. Chem.* 269, 30753-30756.
187. Yokote, K., Mori, S., Hansen, K., McGlade, J., Pawson, T., Heldin, C.-H., and Claesson-Welsh, L. (1994). Direct interaction between Shc and the platelet-derived growth factor β -receptor. *J. Biol. Chem.* 269, 15337-15343.
188. Vassbotn, F.S., Havnen, O.K., Heldin, C.-H., and Holmsen, H. (1994). Negative feedback regulation of human platelets via autocrine activation of the platelet-derived growth factor α -receptor. *J. Biol. Chem.* 269, 13874-13879.
189. Saras, J., Claesson-Welsh, L., Heldin, C.-H., and Gonez, L.J. (1994). Cloning and characterization of PTPL1, a protein tyrosine phosphatase with similarities to cytoskeletal-associated proteins. *J. Biol. Chem.* 269, 24082-24089.
190. Waltenberger, J., Claesson-Welsh, L., Siegbahn, A., Shibuya, M., and Heldin, C.-H. (1994). Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. *J. Biol. Chem.* 269, 26988-26995.
191. Yamashita, H., ten Dijke, P., Franzén, P., Miyazono, K., and Heldin, C.-H. (1994). Formation of hetero-oligomeric complexes of type I and type II receptors for transforming growth factor- β . *J. Biol. Chem.* 269, 20172-20178.
192. Morén, A., Olofsson, A., Stenman, G., Sahlin, P., Kanzaki, T., Claesson-Welsh, L., ten Dijke, P., Miyazono, K., and Heldin, C.-H. (1994). Identification and characterization of LTBP-2, a novel latent transforming growth factor- β -binding protein. *J. Biol. Chem.* 269, 32469-32478.
193. ten Dijke, P., Yamashita, H., Sampath, T.K., Reddi, A.H., Estevez, M., Riddle, D.L., Ichijo, H., Heldin, C.-H., and Miyazono, K. (1994). Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. *J. Biol. Chem.* 269, 16985-16988.
194. Kovalenko, M., Gazit, A., Böhmer, A., Rorsman, C., Rönnstrand, L., Heldin, C.-H., Waltenberger, J., Böhmer, F.-D., and Levitzki, A. (1994). Selective platelet-derived growth factor receptor kinase blockers reverse *sis*-transformation. *Cancer Res.* 54, 6106-6114.
195. Yamashita, H., Okadome, T., Franzén, P., ten Dijke, P., Heldin, C.-H., and Miyazono, K. (1995). A rat pituitary tumor cell line (GH₃) expresses type I and type II receptors and other cell surface binding protein(s) for transforming growth factor- β . *J. Biol. Chem.* 270, 770-774.

196. Eriksson, A., Nånberg, E., Rönstrand, L., Engström, U., Hellman, U., Rupp, E., Carpenter, G., Heldin, C.-H., and Claesson-Welsh, L. (1995). Demonstration of functionally different interactions between phospholipase C- γ and the two types of platelet-derived growth factor receptors. *J. Biol. Chem.* 270, 7773-7781.
197. Franzén, P., Heldin, C.-H., and Miyazono, K. (1995). The GS domain of the transforming growth factor- β type I receptor is important in signal transduction. *Biochem. Biophys. Res. Commun.* 207, 682-689.
198. Kato, M., Ishizaki, A., Hellman, U., Wernstedt, C., Kyogoku, M., Miyazono, K., Heldin, C.-H., and Funahashi, K. (1995). A human keratinocyte cell line produces two autocrine growth inhibitors, transforming growth factor- β and insulin-like growth factor binding protein-6, in a calcium- and cell density-dependent manner. *J. Biol. Chem.* 270, 12373-12379.
199. Hellman, U., Wernstedt, C., Góñez, J., and Heldin, C.-H. (1995). Improvement of an "in-gel" digestion procedure for the micropreparation of internal protein fragments for amino acid sequencing. *Anal. Biochem.* 224, 451-455.
200. Valgeirsdóttir, S., Eriksson, A., Nistér, M., Heldin, C.-H., Westermarck, B., and Claesson-Welsh, L. (1995). Compartmentalization of autocrine signal transduction pathways in Sis-transformed NIH 3T3 cells. *J. Biol. Chem.* 270, 10161-10170.
201. Suzuki, M., Asplund, T., Yamashita, H., Heldin, C.-H., and Heldin, P. (1995). Stimulation of hyaluronan biosynthesis by platelet-derived growth factor-BB and transforming growth factor- β 1 involves activation of protein kinase C. *Biochem. J.* 307, 817-821.
202. Yamashita, H., ten Dijke, P., Huylebroeck, D., Sampath, T.K., Andries, M., Smith, J., C., Heldin, C.-H., and Miyazono, K. (1995). Osteogenic protein-1 binds to activin type II receptors and induces certain activin-like effects. *J. Cell Biol.* 130, 217-226.
203. Andersson, M., Östman, A., Kreysing, J., Bäckström, G., van de Poll, M., and Heldin, C.-H. (1995). Involvement of loop 2 of platelet-derived growth factor-AA and -BB in receptor binding. *Growth Factors* 12, 159-164.
204. Pekny, M., Pekna, M., Östman, A., Törnelli, J., Feinstein, R., Forsberg-Nilsson, K., Heldin, C.-H., Westermarck, B., and Betsholtz, C. (1995). Luteal failure in transgenic mice carrying a PDGF dominant-negative mutant/GH hybrid transgene. *Transgenics* 1, 515-523.
205. Blume-Jensen, P., Wernstedt, C., Heldin, C.-H., and Rönstrand, L. (1995). Identification of the major phosphorylation sites for protein kinase C in Kit/stem cell factor receptor *in vitro* and in intact cells. *J. Biol. Chem.* 270, 14192-14200.
206. Eccleston, P.A., Funahashi, K., and Heldin, C.-H. (1995). Neurons of the peripheral nervous system express thymidine phosphorylase. *Neurosci. Lett.* 192, 137-141.
207. Verschueren, K., Dewulf, N., Goumans, M.-J., Lonnoy, O., Feijen, A., Grimsby, S., Vande Spiegle, K., ten Dijke, P., Morén, A., Vanscheeuwijck, P., Heldin, C.-H., Miyazono, K., Mummery, C., van den Eijnden-van Raaij, J., and Huylebroeck, D. (1995). Expression of type I and type IB receptors for activin in midgestation mouse embryos suggests distinct functions in organogenesis. *Mechanisms of Development* 52, 109-123.
208. Yamada, N., Kato, M., Yamashita, H., Nistér, M., Miyazono, K., Heldin, C.-H., and Funahashi, K. (1995). Enhanced expression of transforming growth factor- β and its type-I and type-II receptors in human glioblastoma. *Int. J. Cancer* 62, 386-392.

209. Rosenzweig, B.L., Imamura, T., Okadome, T., Cox, G.N., Yamashita, H., ten Dijke, P., Heldin, C.-H., and Miyazono, K. (1995). Cloning and characterization of a human type II receptor for bone morphogenetic proteins. *Proc. Natl. Acad. Sci. USA* 92, 7632-7636.
210. Olofsson, A., Ichijo, H., Morén, A., ten Dijke, P., Miyazono, K., and Heldin, C.-H. (1995). Efficient association of an amino-terminally extended form of human latent transforming growth factor- β binding protein with the extracellular matrix. *J. Biol. Chem.* 270, 31294-31297.
211. Nishiyama, A., Lin, X.-H., Giese, N., Heldin, C.-H., and Stallcup, W.B. (1996). Co-localization of NG2 proteoglycan and PDGF α -receptor on O2A progenitor cells in the developing rat brain. *J. Neurosci. Res.* 43, 299-314.
212. Nishiyama, A., Lin, X.-H., Giese, N., Heldin, C.-H., and Stallcup, W.B. (1996). Interaction between NG2 proteoglycan and PDGF α -receptor on O2A progenitor cells is required for optimal response to PDGF. *J. Neurosci. Res.* 43, 315-330.
213. Hermanson, M., Funai, K., Koopmann, J., Maintz, D., Waha, A., Westermarck, B., Heldin, C.-H., Wiestler, O.D., Louis, D.N., von Deimling, A., and Nistér, M. (1996). Association of loss of heterozygosity on chromosome 17p with high platelet-derived growth factor α receptor expression in human malignant gliomas. *Cancer Res.* 56, 164-171.
214. Yokote, K., Margolis, B., Heldin, C.-H., and Claesson-Welsh, L. (1996). Grb7 is a downstream signaling component of platelet-derived growth factor α - and β -receptors. *J. Biol. Chem.* 271, 30942-30949.
215. Green, L.S., Jellinek, D., Jenison, R., Östman, A., Heldin, C.-H., and Janjic, N. (1996). Inhibitory DNA ligands to platelet-derived growth factor B-chain. *Biochem.* 35, 14413-14424.
216. Yamada, N., Kato, M., ten Dijke, P., Yamashita, H., Sampath, T.K., Heldin, C.-H., Miyazono, K., and Funai, K. (1996). Bone morphogenetic protein type IB receptor is progressively expressed in malignant glioma tumours. *Br. J. Cancer* 73, 624-629.
217. Yokote, K., Mori, S., Siegbahn, A., Rönnstrand, L., Wernstedt, C., Heldin, C.-H., and Claesson-Welsh, L. (1996). Structural determinants in the platelet-derived growth factor α -receptor implicated in modulation of chemotaxis. *J. Biol. Chem.* 271, 5101-5111.
218. Soucheinytskyi, S., ten Dijke, P., Miyazono, K., and Heldin, C.-H. (1996). Phosphorylation of Ser165 in TGF- β type I receptor modulates TGF- β 1-induced cellular responses. *EMBO J.* 15, 6231-6240.
219. Hulth, A., Johnell, O., Miyazono, K., Lindberg, L., Heinegård, D., and Heldin, C.-H. (1996). Effect of transforming growth factor- β and platelet-derived growth factor-BB on articular cartilage in rats. *J. Orthop. Res.* 14, 547-553.
220. Hansen, K., Johnell, M., Siegbahn, A., Rorsman, C., Engström, U., Wernstedt, C., Heldin, C.-H., and Rönnstrand, L. (1996). Mutation of a Src phosphorylation site in the PDGF β -receptor leads to increased PDGF-stimulated chemotaxis but decreased mitogenesis. *EMBO J.* 15, 5299-5313.
221. Kreysing, J., Östman, A., van de Poll, M., Bäckström, G., and Heldin, C.-H. (1996). Identification of three amino acid residues in the B-chain of platelet-derived growth factor with different importance for binding to PDGF α - and β -receptors. *FEBS Lett.* 385, 181-184.
222. Omura, T., Heldin, C.-H., and Östman, A. (1997). Immunoglobulin-like domain 4-mediated receptor-receptor interactions contribute to platelet-derived growth factor-induced receptor dimerization. *J. Biol. Chem.* 272, 12676-12682.

223. Hooshmand-Rad, R., Claesson-Welsh, L., Wennström, S., Yokote, K., Siegbahn, A., and Heldin, C.-H. (1997). Involvement of phosphatidylinositol 3'-kinase and Rac in platelet-derived growth factor-induced actin reorganization and chemotaxis. *Exp. Cell Res.* (in press).
224. Hansen, K., Alonso, G., Courtneidge, S.A., Rönstrand, L., and Heldin, C.-H. (1997). PDGF-induced phosphorylation of Tyr28 in the N-terminus of Fyn affects Fyn activation. *Biochem. J.* (submitted).
225. Olofsson, A., Hellman, U., ten Dijke, P., Grimsby, S., Ichijo, H., Morén, A., Miyazono, K., and Heldin, C.-H. (1997). A latent transforming growth factor- β complex in Chinese hamster ovary cells contains the multifunctional cysteine-rich fibroblast growth factor receptor, also termed E-selectin-ligand or MG-160. *Biochem. J.* (in press).
226. Kozawa, O., Blume-Jensen, P., Heldin, C.-H., and Rönstrand, L. (1997). Involvement of phosphatidylinositol 3'-kinase in stem cell factor-induced phospholipase D activation and arachidonic acid release. *Eur. J. Biochem.* (submitted).
227. Hansen, K., Rönstrand, L., Rorsman, C., Hellman, U., and Heldin, C.-H. (1997). Association of coatamer proteins with the β -receptor for platelet-derived growth factor. *Biochem. Biophys. Res. Commun.* (in press).
228. Valgeirsdóttir, S., Paukku, K., Silvennoinen, O., Heldin, C.-H., and Claesson-Welsh, L. (1997). Activation of Stat5 by platelet-derived growth factor (PDGF) is dependent on phosphorylation sites in PDGF β -receptor juxtamembrane and kinase insert domains. *Oncogene* (submitted).
229. Nakao, A., Röijer, E., Imamura, T., Souchelnytskyi, S., Stenman, G., Heldin, C.-H., and ten Dijke, P. (1997). Identification of Smad2, a human Mad-related protein in the transforming growth factor β signaling pathway. *J. Biol. Chem.* 272, 2896-2900.
230. Omura, T., Miyazono, K., Östman, A., and Heldin, C.-H. (1997). Identification of a 190 kDa VEGF₁₆₅ cell surface binding protein on a human glioma cell line. *J. Biol. Chem.* (submitted).
231. Saras, J., Franzén, P., Aspenström, P., Hellman, U., Góñez, L.J., and Heldin, C.-H. (1997). A novel RhoGAP interacts with a PDZ domain of the protein tyrosine phosphatase PTPL1. *J. Biol. Chem.* (submitted).
232. Kovalenko, M., Rönstrand, L., Heldin, C.-H., Gazit, A., Levitzki, A., Loubchenkov, M., and Böhmmer, F.-D. (1997). Phosphorylation site-specific inhibition of platelet-derived growth factor receptor autophosphorylation by the receptor blocking tyrphostin AG1296. *Biochemistry* (in press).
233. Piek, E., Franzén, P., Heldin, C.-H., and ten Dijke, P. (1997). Characterization of a 60 kDa cell surface-associated TGF- β binding that can inhibit TGF- β receptor binding. *J. Cell. Physiol.* (in press).
234. Hansen, K., Rönstrand, L., Claesson-Welsh, L., and Heldin, C.-H. (1997). Phosphorylation of a 72 kDa protein in PDGF-stimulated cells which forms complex with c-Crk, c-Fyn and Eps15. *FEBS Lett.* (in press).
235. Saras, J., Engström, U., Góñez, J., and Heldin, C.-H. (1997). Characterization of the interactions between PDZ domains of PTPL1 and the C-terminal tail of Fas. *J. Biol. Chem.* (submitted).
236. Yokote, K., Hellman, U., Saito, Y., Ekman, S., Rönstrand, L., Saito, Y., Heldin, C.-H., and Mori, S. (1997). Identification of Tyr-762 in the platelet-derived growth factor α -receptor as the binding site for Crk proteins. *Mol. Cell. Biol.* (submitted).

237. Nakao, A., Imamura, T., Souchelnytskyi, S., Kawabata, M., Ishisaki, A., Oeda, E., Tamaki, K., Hanai, J.-i., Heldin, C.-H., Miyazono, K., and ten Dijke, P. (1997). Heteromeric complex formation and activation of Smad2, Smad3 and Smad4 in TGF- β receptor-mediated signaling. *EMBO J.* (submitted).
238. Persson, U., Souchelnytskyi, S., Franzén, P., Miyazono, K., ten Dijke, P., and Heldin, C.-H. (1997). TGF- β -specific signaling by chimeric TGF- β type II receptor with intracellular domain of activin type IIB receptor. *J. Biol. Chem.* (submitted).
239. Souchelnytskyi, S., Tamaki, K., Engström, U., Wernstedt, C., ten Dijke, P., and Heldin, C.-H. (1997). Phosphorylation of Ser465 and Ser467 in the C-terminus of Smad2 is required for TGF- β signalling and mediates direct interaction with Smad4. *EMBO J.* (submitted).
240. Ruusala, A., Sundberg, C., Arvidsson, A.-K., Rupp-Thureson, E., Heldin, C.-H., and Claesson-Welsh, L. (1997). Platelet-derived growth factor (PDGF)-induced actin rearrangement is deregulated in cells expressing a mutant Y778F PDGF β -receptor. *J. Cell Sci.* (submitted).
241. Nakao, A., Morén, A., Heuchel, R., Itoh, S., Heldin, C.-H., and ten Dijke, P. (1997). Identification of Smad7, an inhibitor of TGF- β -induced signaling responses. *Nature* (submitted).
242. Shimizu, A., Kato, M., Nakao, A., ten Dijke, P., Heldin, C.-H., Imamura, T., Kawabata, M., Shimada, S., and Miyazono, K. (1997). Identification of receptors and Smad proteins involved in activin-induced differentiation of human epidermal keratinocytes. *J. Biol. Chem.* (submitted).

REVIEW ARTICLES

1997-05-28

1. Wasteson, Å., Amadò, R., Ingmar, B., and Heldin, C.-H. (1975). Degradation of chondroitin sulphate by lysosomal enzymes from embryonic chick cartilage. In: *Protides of the Biological Fluids* (Peeters, H., eds.), Pergamon Press, New York. Vol. 22, pp. 431-435.
2. Heldin, C.-H., Westermark, B., and Wasteson, Å. (1979). Purification and characterization of human growth factors. In: *Hormones and Cell Culture* (Sato, G.H., and Ross, R., eds.), Cold Spring Harbor Conferences on Cell Proliferation, Vol. 6, pp. 17-31.
3. Busch, C., Ljungman, C., Heldin, C.-H., Wasteson, E., and Öbrink, B. (1979). Surface properties of cultured endothelial cells. In: *Haemostasis* (Brakman, P., eds.), S. Karger, Basel. Vol. 8, pp. 142-148.
4. Heldin, C.-H. (1980). Studies on growth factors for human cultured cells. *Acta Universitatis Upsaliensis, Abstracts of Uppsala Dissertations from the Faculty of Medicine* 359, 1-44.
5. Heldin, C.-H., Wasteson, Å., and Westermark, B. (1981). Specific binding of ¹²⁵I-labeled platelet-derived growth factor to cultured cells. In: *The Biology of Normal Human Growth* (Ritzén, M., et al., eds.), Raven Press, Vol. pp. 23-31.
6. Nistér, M., Heldin, C.-H., Wasteson, Å., and Westermark, B. (1982). A platelet-derived growth factor analog produced by a human clonal glioma cell line. *Ann. N.Y. Acad. Sci.* 397, 25-33.
7. Westermark, B., Heldin, C.-H., Ek, B., Johnsson, A., Mellström, K., Nistér, M., and Wasteson, Å. (1983). Biochemistry and biology of platelet-derived growth factor. In: *Growth and Maturation Factors* (Guroff, G., eds.), John Wiley & Sons, Inc., New York. Vol. 1, pp. 73-115.
8. Heldin, C.-H., Westermark, B., Mellström, K., Johnsson, A., Ek, B., Nistér, M., Betsholtz, C., Rönnstrand, L., and Wasteson, Å. (1983). Platelet-derived growth factor: Structural and functional aspects. In: *Survey and Synthesis of Pathology Research* (eds.), S. Karger AG, Basel. Vol. 1, pp. 153-164.
9. Heldin, C.-H., Ek, B., and Rönnstrand, L. (1983). Characterization of the fibroblast receptor for platelet-derived growth factor. *Cell Biology International Reports* 7, 543-544.
10. Wasteson, Å., Heldin, C.-H., and Westermark, B. (1983). Platelet-derived growth factor: Studier av en tillväxtfaktors verkningsmekanism och funktion. *MFR informerar* 4, no 1, 4-7.
11. Betsholtz, C., Heldin, C.-H., Wasteson, Å., and Westermark, B. (1983). Pure platelet-derived growth factor stimulates human fibroblasts to proliferate in plasma-free culture. In: *Hormonally Defined Media* (Fischer, G., and Wieser, R.J., eds.), Proceedings of Life Sciences, Springer-Verlag, Heidelberg. Vol. pp. 103-105.
12. Waterfield, M.D., Scrace, G.T., Whittle, N., Stockwell, P.A., Stroobant, P., Johnsson, A., Wasteson, Å., Westermark, B., Heldin, C.-H., Huang, J.S., and Deuel, T.F. (1984). Relationship between the transforming protein of simian sarcoma virus and platelet-derived growth factor. In: *Cancer Cells* (eds.), Cold Spring Harbor Laboratories, New York. Vol. 1, pp. 25-33.
13. Westermark, B., Wasteson, Å., and Heldin, C.-H. (1984). Platelet-derived growth factor. In: *Hormones and Cell Regulation* (Dumont, J.E., and Nunez, J., eds.), Elsevier Science Publishers, Amsterdam. Vol. 8, pp. 9-16.

14. Ek, B., Rönstrand, L., and Heldin, C.-H. (1984). Stimulation of tyrosine phosphorylation by platelet-derived growth factor. *Biochem. Soc. Trans.* 12, 759-762.
15. Heldin, C.-H., Wasteson, Å., and Westermark, B. (1984). Platelet-derived growth factor vid normal och malign celltillväxt. *Läkartidningen* 81, 3107-3109 och *Nordisk Medicin* 99, 319-322.
16. Heldin, C.-H., and Westermark, B. (1984). Growth factors: Mechanism of action and relation to oncogenes. *Cell* 37, 9-20.
17. Heldin, C.-H., Wasteson, Å., and Westermark, B. (1985). Platelet-derived growth factor. *Mol. Cell. Endocrinol.* 39, 169-187.
18. Westermark, B., Johnsson, A., Betsholtz, C., Wasteson, Å., and Heldin, C.-H. (1985). Platelet-derived growth factor: Comments on the structural and functional relationship with the transforming protein of simian sarcoma virus. In: *Molecular Biology of Tumor Cells* (Wahren, B., et al., eds.), Raven Press, New York. pp. 87-94.
19. Heldin, C.-H., Betsholtz, C., Johnsson, A., Nistér, M., Ek, B., Rönstrand, L., Wasteson, Å., and Westermark, B. (1985). Platelet-derived growth factor: Mechanism of action and relation to oncogenes. *J. Cell Sci. Suppl.* 3, 65-76.
20. Heldin, C.-H. (1985). Tillväxtfaktorers verkningsmekanism - En nyckel till onkogenproduktens funktion? *Kungl. Vetenskaps-Societetens (Uppsala) Årsbok* 37-41.
21. Westermark, B., Nistér, M., and Heldin, C.-H. (1985). Growth factors and oncogenes in human malignant glioma. In: *Neurologic Clinics* (Vick, N.A., and Bigner, D.D., eds.), W.B. Saunders, Philadelphia. Vol. 3, pp. 785-799.
22. Westermark, B., and Heldin, C.-H. (1985). Platelet-derived growth factor in normal and pathological cell growth. In: *Inflammation Basic Mechanisms, Tissue Injury Principles and Clinical Models* (Venge, P., and Lindbom, A., eds.), Almqvist and Wiksell International, Vol. pp. 255-270.
23. Heldin, C.-H., Wasteson, Å., and Westermark, B. (1986). Platelet-derived growth factor, its receptor and mechanism of action. In: *Mechanisms of Insulin Action* (Belfrage, P., Donner, J., and Strålfors, P., eds.), The Fernström Foundation Series, Elsevier Science Publishers (Biomedical Division), Amsterdam. Vol. 7, pp. 109-121.
24. Westermark, B., and Heldin, C.-H. (1986). Platelet-derived growth factor as a mediator of normal and neoplastic cell proliferation. *Med. Oncol. & Tumor Pharmacother.* 3, 177-183.
25. Heldin, C.-H., Betsholtz, C., Johnsson, A., and Westermark, B. (1986). Role of PDGF-like growth factors in malignant transformation. *Cancer Rev.* 2, 34-47.
26. Heldin, C.-H., and Westermark, B. (1986). Role of PDGF-like growth factors in autocrine stimulation of growth of normal and transformed cells. In: *Oncogenes and Growth Control* (Kahn, P., and Graf, T., eds.), Springer-Verlag, Heidelberg. pp. 43-50.
27. Heldin, C.-H., and Westermark, B. (1986). Platelet-derived growth factor: Structure, function, and role in autocrine stimulation of growth. In: *Cell Cycle and Oncogenes* (Gallwitz, D., and Tanner, W., eds.), Springer-Verlag, Heidelberg. pp. 137-144.
28. Westermark, B., Johnsson, A., Betsholtz, C., and Heldin, C.-H. (1987). Biological properties of simian sarcoma virus and its oncogene product. In: *Contr. Oncol.* eds.), Karger, Basel. Vol. 24, pp. 51-61.

29. Heldin, C.-H., Johnsson, A., Ek, B., Wennergren, S., Rönstrand, L., Hammacher, A., Faulders, B., Wasteson, A., and Westermark, B. (1987). Purification of human platelet-derived growth factor. *Meth. Enzymol.* 147, 3-13.
30. Westermark, B., Betsholtz, C., Johnsson, A., and Heldin, C.-H. (1987). Acute transformation by simian sarcoma virus is mediated by an externalized PDGF-like growth factor. In: *Viral Carcinogenesis* (Kjeldgaard, N.O., and Forchhammer, J., eds.), Munksgaard, Copenhagen. Vol. pp. 445-457.
31. Fellström, B., Klareskog, L., Larsson, E., Tufveson, G., Wahlberg, J., Rönstrand, L., Heldin, C.-H., Terracio, L., and Rubin, K. (1987). Tissue distribution of macrophages, class II transplantation antigens, and receptors for platelet-derived growth factor in normal and rejected human kidneys. *Transpl. Proc.* 19, 3625-3627.
32. Westermark, B., and Heldin, C.-H. (1987). Platelet-derived growth factor. In: *Highlights in Endocrinology* (Christiansen, C., and Juel Riis, B., eds.), Munksgaard, Copenhagen. Vol. pp. 211-216.
33. Westermark, B., and Heldin, C.-H. (1987). Structure and function of platelet-derived growth factor. *Acta Med. Scand., Suppl.* 715, 19-23.
34. Heldin, C.-H., and Westermark, B. (1987). PDGF-like growth factors in autocrine stimulation of growth. *J. Cell. Phys. Suppl.* 5, 31-34.
35. Heldin, C.-H., Betsholtz, C., Claesson-Welsh, L., and Westermark, B. (1987). Subversion of growth regulatory pathways in malignant transformation. *Biochim. Biophys. Acta* 907, 219-244.
36. Westermark, B., Nistér, M., and Heldin, C.-H. (1987). Oncogenes, growth factors and the pathogenesis of human glioma: The 1986 Engelhardt lecture. In: *Brain Oncology* (Chatel, M., Darcel, F., and Pecker, J., eds.), Martinus Nijhoff Publishers, Dordrecht. Vol. pp. 7-13.
37. Westermark, B., and Heldin, C.-H. (1987). Platelet-derived growth factor: structure, function and biological responses. In: *Tissue Fibrosis: Immune Cells and Mediators, Local Immunity* (Revillard, J.-P., and Wierzbicki, N., eds.), Fondation Franco-Allemande, Suresnes, France. Vol. 3, pp. 73-83.
38. Heldin, C.-H., and Westermark, B. (1987). Role of PDGF-like growth factors in cell transformation. In: *Theories of Carcinogenesis* (Iversen, O.H., eds.), Hemisphere Publishing Corporation, Washington. Vol. pp. 81-91.
39. Westermark, B., and Heldin, C.-H. (1988). Activation of proto-oncogenes coding for growth factors or growth factor receptors. In: *Cellular Oncogene Activation* (Klein, G., eds.), Marcel Dekker, Inc., New York. Vol. pp. 149-180.
40. Claesson-Welsh, L., Eriksson, A., Severinsson, L., Westermark, B., and Heldin, C.-H. (1988). Structural features of A and B type PDGF receptors. In: *Progress in Endocrinology 1988* (Imura, H., et al., eds.), Elsevier Science Publishers B.V. (Biomedical Division), Vol. pp. 503-508.
41. Heldin, C.-H., and Westermark, B. (1988). Tillväxtfaktorer och onkgener. *Läkartidningen* 85, 497-499.
42. Heldin, C.-H., Hammacher, A., Nistér, M., and Westermark, B. (1988). Structural and functional aspects of platelet-derived growth factor. *Br. J. Cancer* 57, 591-593.
43. Heldin, C.-H., and Westermark, B. (1988). Platelet-derived growth factor and its relation to oncogenes. *ISI Atlas of Science: Immunology* 44-48.

44. Westermark, B., Betsholtz, C., Claesson-Welsh, L., Nistér, M., and Heldin, C.-H. (1988). Molecular mimicry within the platelet-derived growth factor family: Identification of three isoforms that bind to two distinct but related cell surface receptors. In: *Molecular Mimicry in Health and Disease* (Lernmark, Å., Dyrberg, T., Terenius, L., and Hökfelt, B., eds.), Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam. Vol. pp. 135-144.
45. Westermark, B., and Heldin, C.-H. (1989). Platelet-derived growth factor: structural and functional aspects. *J. Internal Medicine* 225, 55-58.
46. Heldin, C.-H., and Rönstrand, L. (1989). The platelet-derived growth factor receptor. In: *Receptor Phosphorylation* (Moudgil, V.K., eds.), CRC Press, Boca Raton, FL. Vol. pp. 149-162.
47. Westermark, B., Claesson-Welsh, L., and Heldin, C.-H. (1989). Structural and functional aspects of the receptors for platelet-derived growth factor. *Prog. Growth Factor Res.* 1, 253-266.
48. Heldin, C.-H., and Westermark, B. (1989). Growth factors as transforming proteins. *Eur. J. Biochem.* 184, 487-496.
49. Betsholtz, C., Rorsman, F., Bywater, M., Heldin, C.-H., and Westermark, B. (1989). Platelet-derived growth factor - Structural and functional aspects of the A-chain gene. In: *Advances in Growth Hormone and Growth Factor Research* (Müller, E.E., Cocchi, D., and Locatelli, V., eds.), Pythagora Press, Roma-Milano and Springer Verlag, Heidelberg. Vol. pp. 181-190.
50. Westermark, B., and Heldin, C.-H. (1989). Platelet-derived growth factor and its role in mitogenesis and transformation. In: *Cell to Cell Signals in Mammalian Development* (de Laat, S.W., et al., eds.), NATO ASI Series, Springer-Verlag, Heidelberg. Vol. H26, pp. 261-269.
51. Heldin, C.-H., and Westermark, B. (1989). Platelet-derived growth factor: Three isoforms and two receptor types. *Trends in Genetics* 5, 108-111.
52. Heldin, C.-H., and Westermark, B. (1989). Platelet-derived growth factors: A family of isoforms that bind to two distinct receptors. In: *Growth Factors. British Medical Bulletin* (Waterfield, M., eds.), Churchill Livingstone, Vol. 45, pp. 453-464.
53. Claesson-Welsh, L., and Heldin, C.-H. (1989). Platelet-derived growth factor. Three isoforms that bind to two distinct cell surface receptors. *Acta Oncologica* 28, 331-334.
54. Heldin, C.-H., and Westermark, B. (1989). Wachstumsfaktoren und Zelltransformation. In: *Endokrinologie* (Hesch, H.D., eds.), Urban und Schwarzenberg, München. Vol. pp. 370-374.
55. Nistér, M., Betsholtz, C., Heldin, C.-H., and Westermark, B. (1989). Platelet-derived growth factor. In: *Growth Factors and Oncogenes* (Bolla, M., Chambaz, E.M., and Vrousos, C., eds.), Colloque INSERM/John Libbey Eurotext Ltd., Vol. 190, pp. 25-33.
56. Betsholtz, C., Nistér, M., Rorsman, F., Heldin, C.-H., and Westermark, B. (1989). Structural and functional aspects of platelet-derived growth factor and its role in the pathogenesis of glioblastoma. *Mol. Chem. Neuropath.* 10, 27-36.
57. Westermark, B., and Heldin, C.-H. (1989). Growth factors and their receptors. *Current Opinion in Cell Biology* 1, 279-285.
58. Westermark, B., Nistér, M., Heldin, N.-E., and Heldin, C.-H. (1990). Oncogene expression and control of growth in malignant brain tumours. In: *Neuro-oncology. Primary malignant brain tumours* (Thomas, D.G.T., eds.), Edward Arnold Publishers, London. Vol. pp. 26-39.

59. Heldin, C.-H., and Westermark, B. (1990). Autocrine stimulation of growth of normal and transformed cells. In: *Growth Factors, Differentiation Factors, and Cytokines* (Habenicht, A., eds.), Springer-Verlag, Heidelberg. Vol. pp. 267-278.
60. Heldin, C.-H., and Westermark, B. (1990). Role of platelet-derived growth factor in autocrine and paracrine stimulation of normal and malignant cells. In: *Malignant Cell Secretion* (Krsmanovic, V., and Whitfield, J.F., eds.), CRC Press, Inc., Boca Raton, FL. Vol. pp. 45-56.
61. Heldin, C.-H., and Westermark, B. (1990). Growth factors and their receptors. In: *Handbook of Experimental Pharmacology, Carcinogenesis and Mutagenesis* (Cooper, C.S., and Grover, P.L., eds.), Springer Verlag, Heidelberg. Vol. 94 part II, pp. 353-379.
62. Miyazono, K., and Heldin, C.-H. (1990). Platelet-derived endothelial cell growth factor. In: *Handbook of Experimental Pharmacology, Peptide Growth Factors and Their Receptors II* (Sporn, M.B., and Roberts, A.B., eds.), Springer-Verlag, Heidelberg. Vol. 95/II, pp. 125-133.
63. Miyazono, K., Yuki, K., Takaku, F., Wernstedt, C., Kanzaki, T., Olofsson, A., Hellman, U., and Heldin, C.-H. (1990). Latent Forms of TGF- β : Structure and Biology. *Ann. N.Y. Acad. Sci.* 593, 51-58.
64. Heldin, C.-H., Claesson-Welsh, L., and Westermark, B. (1990). Platelet-derived growth factor and its receptors. In: *Growth Factors: From Genes to Clinical Application* (Sara, V.R., et al., eds.), Raven Press, New York. Vol. pp. 41-50.
65. Heldin, C.-H., and Rönstrand, L. (1990). Platelet-derived growth factor B type receptor. In: *Receptor Purification* (Litwack, G., eds.), Humana Press, Clifton, NJ. Vol. 1, pp. 303-314.
66. Heldin, C.-H., Miyazono, K., Claesson-Welsh, L., and Westermark, B. (1990). Two platelet-derived mitogens with potentially different roles in vessel wall biology. In: *Applied Cardiovascular Biology 1989* (Zilla, P., Fasol, R., and Callow, A., eds.), Karger, S., Basel. Vol. 1, pp. 12-21.
67. Westermark, B., Claesson-Welsh, L., and Heldin, C.-H. (1990). Structural and functional aspects of platelet-derived growth factor and its receptors. *Proceedings of Ciba Foundation Symposium no 150 on Proto-oncogenes in Cell Development*. Wiley J. & Sons, Chichester, pp. 6-22.
68. Kriz, R., Lin, L.-L., Sultzman, L., Ellis, C., Heldin, C.-H., Pawson, T., and Knopf, J. (1990). Phospholipase C isozymes: structural and functional similarities. *Proceedings of Ciba Foundation Symposium no 150 on Proto-oncogenes in Cell Development*. Wiley, J. & Sons, Chichester, pp. 112-127.
69. Heldin, C.-H., and Westermark, B. (1990). Signal transduction by the receptors for platelet-derived growth factor. *J. Cell Sci.* 96, 193-196.
70. Betsholtz, C., Rorsman, F., Westermark, B., Östman, A., and Heldin, C.-H. (1990). Analogous alternative splicing. *Nature* 344, 299.
71. Heldin, C.-H., and Westermark, B. (1990). Platelet-derived growth factor: mechanism of action and possible in vivo function. *Cell Regulation* 1, 555-566.
72. Heldin, C.-H., Miyazono, K., Claesson-Welsh, L., and Westermark, B. (1990). Growth regulatory proteins from human platelets. In: *Genetic Basis for Carcinogenesis: Tumor Suppressor Genes and Oncogenes* (Knudson, A.G., Jr., et al., eds.), Japan Sci. Soc. Press, Tokyo/Taylor & Francis Ltd., London, pp. 81-92.

73. Heldin, C.-H., and Westermark, B. (1990). Structural and functional aspects of platelet-derived growth factor and its receptors. In: *Oncogenes in Cancer Diagnostics, Contrib. Oncol.* (Bartram, C.R., Munk, K., and Schwab, M., eds.), Karger, Basel. Vol. 39, pp. 115-124.
74. Betsholtz, C., Nistér, M., Heldin, C.-H., and Westermark, B. (1990). Platelet-derived growth factor: Role in gliogenesis and in the development of glioblastoma. In: *Trophic Factors and the Nervous System* (Horrocks, L.A., et al., eds.), Raven Press, Ltd., New York. Vol. pp. 35-45.
75. Siegbahn, A., Claesson-Welsh, L., Heldin, C.-H., and Westermark, B. (1990). Induction of chemotaxis by PDGF-BB through the B type receptor requires a functional receptor protein tyrosine kinase activity. In: *Molecular and Cellular Biology of Cytokines* (eds.), Wiley-Liss, Inc., Vol. pp. 259-264.
76. Miyazono, K., Usuki, K., and Heldin, C.-H. (1990). Structural and functional properties of platelet-derived endothelial cell growth factor. In: *Growth Factors in Health and Disease* (Westermark, B., Betsholtz, C., and Hökfelt, B., eds.), Elsevier Science Publishers B.V., Vol. pp. 281-288.
77. Heldin, C.-H., and Westermark, B. (1991). Platelet-derived growth factor and autocrine mechanisms of oncogenic processes. In: *CRC Critical Reviews in Oncogenesis* (eds.), CRC Press, Inc., Vol. 2, pp. 109-124.
78. Heldin, C.-H., Hellman, U., Ishikawa, F., and Miyazono, K. (1991). Purification, cloning, and expression of platelet-derived endothelial cell growth factor. In: *Methods in Enzymology. Peptide Growth Factors. Part C* (Barnes, D., Mather, J.P., and Sato, G.H., eds.), Methods in Enzymology, Academic Press, Inc, San Diego. Vol. 198, pp. 383-391.
79. Claesson-Welsh, L., Eriksson, A., Westermark, B., and Heldin, C.-H. (1991). Cloning and expression of human platelet-derived growth factor α and β receptors. In: *Methods in Enzymology. Peptide Growth Factors. Part C* (Barnes, D., Mather, J.P., and Sato, G.H., eds.), Methods in Enzymology, Academic Press, Inc, San Diego. Vol. 198, pp. 72-77.
80. Rönstrand, L., and Heldin, C.-H. (1991). Purification of platelet-derived growth factor β receptor from porcine uterus. In: *Methods in Enzymology* (eds.), Vol. 200, pp. 371-378.
81. Miyazono, K., and Heldin, C.-H. (1991). Latent forms of TGF- β : molecular structure and mechanisms of activation. In: *Proceedings of Ciba Foundation Symposium no 157 on Clinical Applications of TGF- β* (eds.), Wiley, Chichester. Vol. pp. 81-92.
82. Heldin, C.-H., and Westermark, B. (1991). PDGF/PDGF receptor files. *Cancer Cells* 3, 252-254.
83. Heldin, C.-H., Usuki, K., and Miyazono, K. (1991). Platelet-derived endothelial cell growth factor. *J. Cell. Biochem.* 47, 208-210.
84. Westermark, B., and Heldin, C.-H. (1991). Platelet-derived growth factor in autocrine transformation. *Cancer Res.* 51, 5087-5092.
85. Heldin, C.-H. (1991). SH2-domains: elements that control protein interactions during signal transduction. *TIBS* 16, 450-452.
86. Miyazono, K., Usuki, K., and Heldin, C.-H. (1991). Platelet-derived endothelial cell growth factor. *Prog. Growth Factor Res.* 3, 207-217.
87. Heldin, C.-H., Östman, A., Eriksson, A., Siegbahn, A., Claesson-Welsh, L., and Westermark, B. (1992). Platelet-derived growth factor: Isoform-specific signalling via heterodimeric or homodimeric receptor complexes. *Kidney Int.* 41, 571-574.

88. Heldin, C.-H. (1992). Structural and functional studies on platelet-derived growth factor. *EMBO J.* 11, 4251-4259.
89. Miyazono, K., and Heldin, C.-H. (1992). Structure, function and possible clinical application of transforming growth factor- β . *J. Dermatol.* 19, 644-647.
90. Miyazono, K., and Heldin, C.-H. (1993). The mechanism of action of transforming growth factor- β . *Gastroenterologica Japonica* Vol. 28, suppl. 4, pp. 81-85.
91. Miyazono, K., Ichijo, H., and Heldin, C.-H. (1993). Transforming growth factor- β : Latent forms, binding proteins and receptors. *Growth Factors* 8, 11-22.
92. Heldin, C.-H., and Westermark, B. (1993). Possible *in vivo* effect and clinical utility of platelet-derived growth factor (PDGF) and PDGF antagonists. *Transpl. Proc.* 25, 2072-2074.
93. Waltenberger, J., Miyazono, K., Funa, K., Wanders, A., Fellström, B., and Heldin, C.-H. (1993). Transforming growth factor- β and organ transplantation. *Transpl. Proc.* 25, 2038-2040.
94. Heldin, C.-H., Östman, A., and Westermark, B. (1993). Structure of platelet-derived growth factor: Implications for functional properties. *Growth Factors* 8, 245-252.
95. Westermark, B., and Heldin, C.-H. (1993). Platelet-derived growth factor: structure, function and implications in normal and malignant cell growth. *Acta Oncologica* 32, 101-105.
96. Heldin, C.-H. (1993). Purification and structure of PDGF. In: *Biology of platelet-derived growth factor. Cytokines* (Westermark, B., and Sorg, C., eds.), Karger, S. AG, Basel. Vol. 5, pp. 1-10.
97. Heldin, C.-H., Claesson-Welsh, L., Miyazono, K., and Westermark, B. (1993). Growth factors: In vivo function and mechanism of action. In: *Growth Factors and the cardiovascular system* (Cummins, P., ed.), Kluwer Academic Publishers, Dordrecht. pp. 1-15.
98. Kato, M., Hellman, U., Wernstedt, C., Miyazono, K., Heldin, C.-H., and Funa, K. (1994). Characterization of keratinocytes-derived autocrine growth inhibitors. In: *Proceedings of the XVI International Cancer Congress in New Delhi* (Rao, R.S., Deo, M.G., Sanghvi, L.D., and Mittra, I., eds.), Monduzzi Editore, Bologna. pp. 3035-3039.
99. Miyazono, K., ten Dijke, P., Ichijo, H., and Heldin, C.-H. (1994). Receptors for transforming growth factor- β . *Adv. Immunol.* 55, 181-220.
100. ten Dijke, P., Franzén, P., Yamashita, H., Ichijo, H., Heldin, C.-H., and Miyazono, K. (1994). Serine/threonine kinase receptors. *Prog. Growth Factor Res.* 5, 55-72.
101. Miyazono, K., ten Dijke, P., Yamashita, H., and Heldin, C.-H. (1994). Receptors for transforming growth factor- β . In: *Horizon in Cytokine Research. The Third International Mochida Memorial Symposium 1993* (Takaku, F., eds.), Shobunsha Co., Ltd., Tokyo. Vol. pp. 87-96.
102. Miyazono, K., ten Dijke, P., Yamashita, H., and Heldin, C.-H. (1994). Signal transduction via serine/threonine kinase receptors. *Seminars in Cell Biology* 5, 389-398.
103. Heldin, C.-H., and Westermark, B. (1994). Platelet-derived growth factor (PDGF). In: *Guidebook to Cytokines and their Receptors* (Nicola, N., ed.), Oxford University Press, Oxford. pp. 202-204.

104. Heldin, C.-H., and Claesson-Welsh, L. (1994). Receptors for platelet-derived growth factor (PDGF). In: *Guidebook to Cytokines and their Receptors* (Nicola, N., ed.), Oxford University Press, Oxford, pp. 205-207.
105. Westermark, B., Heldin, C.-H., and Nistér, M. (1995). Platelet-derived growth factor in human glioma. *GLIA* 15, 257-263.
106. Heldin, C.-H. (1995). Dimerization of cell surface receptors in signal transduction. *Cell* 80, 213-223.
107. Fredholm, B.B., and Heldin, C.-H. (1995). Ny serie: Tillväxtfaktorer. *Läkartidningen* 92, 1454-1457.
108. Fredholm, B.B., and Heldin, C.-H. (1995). Tillväxtfaktorer - mekanismerna klarnar. *Läkartidningen* 92, 1459-1467.
109. Heldin, C.-H., and Miyazono, K. (1995). Transforming growth factor- β . Intressant kandidat för klinisk användning. *Läkartidningen* 92, 1569-1572.
110. Heldin, C.-H., and Westermark, B. (1996). Role of platelet-derived growth factor *in vivo*. In: *The Molecular and Cellular Biology of Wound Repair* (Clark, R.A.F., ed.), Plenum Press, New York, pp. 249-273.
111. Heldin, C.-H., Östman, A., and Westermark, B. (1996). Platelet-derived growth factor. In: *Growth Factors and Cytokines in Health and Disease* (LeRoith, D., and Bondy, C., eds.), JAI Press Inc., Greenwich, CT, Vol. 1A, pp. 123-145.
112. Yamashita, H., ten Dijke, P., Heldin, C.-H., and Miyazono, K. (1996). Bone morphogenetic protein receptors. *Bone* 19, 569-574.
113. Saras, J., and Heldin, C.-H. (1996). PDZ domain bind carboxy-terminal sequences of target proteins. *Trends. Biol. Sci.* 21, 455-458.
114. Heldin, C.-H. (1996). Protein tyrosine kinase receptors. In: *Cancer Surveys* (Parker, P., and Pawson, T., eds.), Cold Spring Harbor Press, New York, Vol. 27, pp. 7-24.
115. ten Dijke, P., Miyazono, K., and Heldin, C.-H. (1996). Signaling via hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors. *Curr. Opin. Cell Biol.* 8, 139-145.
116. Heldin, C.-H., and Östman, A. (1996). Ligand-induced dimerization of growth factor receptors: variations on the theme. *Cytokines and Growth Factor Reviews* 7, 3-10.
117. Heldin, C.-H., and Purton, M., eds. (1996). Signal Transduction. *Modular Texts in Molecular and Cell Biology*, Chapman and Hall, London.
118. Derynck, R., Gelbart, W.M., Harland, R.M., Heldin, C.-H., Kern, S.E., Massagué, J., Melton, D.A., Mlodzik, M., Padgett, R.W., Roberts, A.B., Smith, J., Thomsen, G.H., Vogelstein, B., and Wang, X.-F. (1996). Nomenclature: Vertebrate mediators of TGF β family signals. *Cell* 87, 173-173.
119. Heldin, C.-H., Claesson-Welsh, L., Miyazono, K., and Westermark, B. (1997). Growth regulatory proteins and their receptors. In: *Encyclopedia of Cancer Biology* (Bertino, J.R., ed.), Academic Press, San Diego, CA, Vol. II, pp. 772-782.
120. Heldin, C.-H., and Rönstrand, L. (1997). Growth factor receptors in cell transformation. In: *Frontiers in Molecular Biology - Oncogenes and Tumor Suppressor Genes* (Peters, G., and Vousden, K., eds.), Oxford University Press, Oxford, (in press).
121. Heldin, C.-H., Miyazono, K., and ten Dijke, P. (1997). Intracellular signaling by TGF- β family members. *Nature* (submitted).

122. Betsholtz, C., and Heldin, C.-H. (1997). Platelet-derived growth factor: A regulator of connective tissue development and reaction. In: *Wennergren Symposia Series*, Portland Press, London, (in press).
123. Miyazono, K., ten Dijke, P., Souchelnytskyi, S., Nakao, A., Imamura, T., Hanai, J.-i., Kawabata, M., and Heldin, C.-H. (1997). Transforming growth factor- β receptors and signal transduction. In: *Serono Symposia on "Inhibin, activin and follistatin; Recent advances and future views"* (Aono, T., Sugino, H., and Vale, W.W., eds.), (in press).
124. Heldin, C.-H. (1997). Simultaneous induction of stimulatory and inhibitory signals by PDGF. *FEBS Lett.* (in press).
125. Heldin, C.-H. (1997). Members of the transforming growth factor- β superfamily signal through serine/threonine kinase receptors. *Japanese J. Cancer Res.* (in press).

Exhibit B

1. (Twice amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase.

2. (Amended) A purified and isolated polypeptide comprising an amino acid sequence shown in SEQ ID NO: 33.

8. (Three times amended) A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding.

9. (Twice amended) A polypeptide according to claim 8 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

12. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

13. A polypeptide according to claim 1 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

14. (Amended) A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

15. A purified and isolated polypeptide according to claim 14, said polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13.

16. (Amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

17. (Twice amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, said polypeptide being purifyable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

19. A polypeptide according to claim 1 further comprising a detectable label.

20. A polypeptide according to claim 8 which is capable of binding the extracellular domain of Flt4 receptor tyrosine kinase with high affinity and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

21. (Amended) A polypeptide according to claim 17 further comprising a detectable label.

22. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 17 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

23. A polypeptide according to claim 14 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

24. A polypeptide according to claim 14 comprising a portion of SEQ ID NO: 33 effective to permit binding to Flt4 receptor tyrosine kinase and stimulation of Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

25. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 16 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

26. A polypeptide according to claim 8 wherein said portion of SEQ ID NO: 33 effective to permit such binding is a continuous portion of SEQ ID NO: 33 within amino acids 1-180 of SEQ ID NO: 33.

27. A polypeptide according to claim 8 wherein the amino terminus of said portion effective to permit such binding corresponds with position 34 of SEQ ID NO: 33.

28. A polypeptide according to claim 16 further comprising a detectable label.



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

DEAFCE:1994

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.

EXAMINER	
Brian Lathrop	
ART UNIT	PAPER NUMBER
1801	21
DATE MAILED:	

Please find below a communication from the EXAMINER in charge of this application
Commissioner of Patents

Priority

1. Applicant correctly contends that the adverse determination of priority under 35 USC 120 is inappropriate in the absence of intervening prior art used in a rejection (part D of Paper No. 20), and it is therefore **moot and withdrawn as premature**. Applicant has met the formal requirements for priority under 35 USC 120 to US Serial No. 08/340011.
2. Receipt of the declaration under 37 CFR 1.132 filed 6/16/97, Paper No. 20, is acknowledged. The declaration has entered but not considered because the adverse determination of priority under 35 USC 120 to which it is addressed is moot.

Double Patenting

3. Co-pending application Serial No. 08/671573, which has recently come to the examiner's attention, claims subject matter which substantially overlaps the instantly claimed subject matter. *New rejections will be set forth in the next office action.* *BT*

Withdrawal of Finality

4. Because new rejections based on the judicially created doctrine of provisional double patenting will be required, the finality of the previous Office action is **withdrawn**.


Entry of Amendment

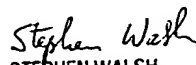
5. Receipt of the amendment filed 6/16/97, Paper No. 20, is acknowledged. The amendment

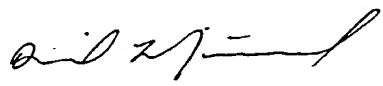
has been entered.

Suspension of Prosecution

6. A reference relevant to the examination of this application may soon become available. *Ex parte* prosecution is SUSPENDED FOR A PERIOD OF 6 MONTHS from the date of this letter. Upon expiration of the period of suspension, applicant should make an inquiry as to the status of the application.


Brian Lathrop, PhD
6/24/97


STEPHEN WALSH
SUPERVISORY PATENT EXAMINER
GROUP 1800


DAVID L. FITZGERALD
PRIMARY EXAMINER
GROUP 1800

Dec. 29. 1997 3:37PM MARSHALL, O'TOOLE

No. 4468 P. 2/2

From: 0808

PATENT APPLICATION
DOCKET NO. 28967/32863

IN THE UNITED STATES PATENT
AND TRADEMARK OFFICE

Application of:)	Certificate of Facsimile Transmittal
)	
Alitalo et al.)	I hereby certify that this paper is being
)	transmitted via facsimile to the Assistant
Serial No: 08/510,133)	Commissioner for Patents, Washington, D.C.
)	20231, on this date:
Filed: August 1, 1995)	
)	Date: <u>Monday, December 29, 1997</u>
For: RECEPTOR LIGAND)	
)	Facsimile No. 1-703-305-7401
Group Art Unit: 1801)	
)	<u>David A. Gass</u>
Examiner: Lathrop, B)	David A. Gass

STATUS INQUIRY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In a communication dated June 25, 1997, the Patent Office suspended prosecution for six months because "a reference relevant to the examination of this application may soon become available." The Applicants were advised to make a status inquiry upon expiration of the six month period. Please advise as to the status of this application at your earliest convenience.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:

David A. Gass
David A. Gass
Reg. No: 38,153

Date:

12/29/97

RECEIVED
DEC 29 1997
GROUP 1800

Dec. 29. 1997 3:37PM MARSHALL, O'TOOLE

No. 4468 P. 1/2
From: 0808

MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BORUN

ATTORNEYS AT LAW
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606-6402
(312) 474-6300
FAX: (312) 474-0448

#22
NB
01/07/96

December 29, 1997

FACSIMILE TRANSMITTAL SHEET

TO: Assistant Commissioner for Patents
1-703-305-7401

CLIENT NO: 28967
MATTER NO: 32863
COUNTRY CODE: US

FROM: David A. Gass

RECEIVED
DEC 29 1997
GROUP 1800

PAGES (INCLUDING THIS PAGE): 2

PLEASE CONFIRM RECEIPT: Yes

MESSAGE:

Please contact Muriel Gallaher at 312-474-6808 if you do not receive all of the pages in good condition.

The material of this transmission contains confidential information intended only for the addressee. If you are not the addressee, any disclosure or use of this information by you is strictly prohibited. If you have received this facsimile in error, please notify us by telephone immediately.



G-111-1801

1252

#27
126
04/10/98

PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Alitalo et al.
Serial No. 08/510,133
Filed: August 1, 1995
For: RECEPTOR LIGAND
Art Unit: 1801
Examiner: Lathrop, B.

) I hereby certify that this paper is
) being deposited with the United
) States Postal Service as first class
) mail, postage prepaid, in an
) envelope addressed to: Assistant
) Commissioner for Patents,
) Washington, D.C. 20231, on this
) date:

) Dated: Feb 24, 1998

) David A. Gass
) David A. Gass

16X-125
NO 1/25

TRANSMITTAL OF POWERS OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are power of attorney documents executed by the two assignees of the above-identified patent application: Helsinki University Licensing, Ltd., and The Ludwig Institute for Cancer Research.

The above-identified application was assigned by the inventors to Helsinki University Licensing, Ltd., (HUL) in an assignment recorded at Reel 8378, Frame 0566.

HUL assigned a 50% interest in the application to The Ludwig Institute for Cancer Research, as evidenced by the attached assignment document which has been submitted for recordation.

Please enter the power of attorney documents into the file for the above-identified patent application.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:

David A. Gass

David A. Gass

Registration No. 38,153

Date: Feb 24, 1998



POWER OF ATTORNEY

The Ludwig Institute for Cancer Research hereby appoints:

Alvin D. Shulman (19,412)
Owen J. Murray (22,111)
Allen H. Gerstein (22,218)
Nate F. Scarpelli (22,320)
Edward M. O'Toole (22,477)
Michael F. Borun (25,447)
Trevor B. Joiko (25,542)

Timothy J. Vezeau (26,348)
Carl E. Moore, Jr. (26,487)
Richard H. Anderson (26,526)
Patrick D. Ertel (26,877)
James P. Zeller (28,491)
William E. McCracken (30,195)
David A. Gass (38,153)

Richard A. Schnurr (30,890)
Anthony Nimmo (30,920)
Christine A. Dudzik (31,245)
Kevin D. Hogg (31,839)
Jeffrey S. Sharp (31,879)
Martin J. Hirsch (32,237)

James J. Napoli (32,361)
Richard M. La Barge (32,254)
Karl A. Vick (33,288)
Douglass C. Hochstetler (33,710)
Cynthia L. Schaller (34,245)
Robert M. Gerstein (34,824)

as its attorneys, with full powers of substitution and revocation, to act on its behalf before the U.S. Patent and Trademark Office in connection with the following applications filed by Kari Alitalo et al. of which it is an assignee:

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>	<u>Assignment Reel & Frame #</u>
08/510,133	01/Aug/95	Receptor Ligand	8378/0566
08/585,895	12/Jan/96	Receptor Ligand	8145/0829
08/601,132	14/Feb/96	Antibodies Reactive with VEGF-C, a Ligand for the Flt4 receptor Tyrosine Kinase (VEGFR-3)	8129/0688
08/671,573	28/Jun/96	Receptor Ligand VEGF-C	8161/0909

Please continue to send correspondence to:

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
United States of America
(312) 474-6300

Ludwig Institute for Cancer Research
1345 Avenue of the Americas
New York, New York 10105

(Date) 26-01-98

By: 

Name: A. Mousa Ntous

Title: ASSOCIATE DIRECTOR



POWER OF ATTORNEY

Helsinki University Licensing, Ltd., hereby appoints:

Alvin D. Shulman (19,412)
Owen J. Murray (22,111)
Allen H. Gerstein (22,218)
Nate F. Scarpelli (22,320)
Edward M. O'Toole (22,477)
Michael F. Borun (25,447)
Trevor B. Joike (25,542)

Timothy J. Vezeau (26,348)
Carl E. Moore, Jr. (26,487)
Richard H. Anderson (26,526)
Patrick D. Ertel (26,877)
James P. Zeller (28,491)
William E. McCracken (30,195)
David A. Gass (38,153)

Richard A. Schnurr (30,890)
Anthony Nimmo (30,920)
Christine A. Dudzik (31,245)
Kevin D. Hogg (31,839)
Jeffrey S. Sharp (31,879)
Martin J. Hirsch (32,237)

James J. Napoli (32,361)
Richard M. La Barge (32,254)
Karl A. Vick (33,288)
Douglass C. Hochstetler (33,710)
Cynthia L. Schaller (34,245)
Robert M. Gerstein (34,824)

as its attorneys, with full powers of substitution and revocation, to act on its behalf before the U.S. Patent and Trademark Office in connection with the following applications filed by Kari Alitalo et al. of which it is an assignee:

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>	<u>Assignment Reel & Frame #</u>
08/510,133	01/Aug/95	Receptor Ligand	8378/0566
08/585,895	12/Jan/96	Receptor Ligand	8145/0829
08/601,132	14/Feb/96	Antibodies Reactive with VEGF-C, a Ligand for the Flt4 Receptor Tyrosine Kinase (VEGFR-3)	8129/0688
08/671,573	28/Jun/96	Receptor Ligand VEGF-C	8161/0909

Please continue to send correspondence to:

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
United States of America
(312) 474-6300

Helsinki University Licensing, Ltd.
Viikinkaari 8 A
FIN-00710 Helsinki
FINLAND

(Date) 28th of June 1998

By:

Name:

Title:

Heikki Lampi

President

ASSIGNMENT

WHEREAS Helsinki University Licensing, Ltd., Viikinkaari 8 A, FIN-00710 Helsinki, Finland (hereinafter HUL), its successors and assigns, is the assignee of the entire right, title and interest in the invention or improvements of Kari Alitalo and Vladimir Joukov relating to the cloning, isolation and sequencing of human Vascular Endothelial Growth Factor C (VEGF-C) disclosed in certain applications for Letters Patent of the United States, and in said applications and any and all other applications, both United States and foreign, which Kari Alitalo and Vladimir Joukov may file, either solely or jointly with others, on said invention or improvements, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said applications, and in any reissue or extension thereof; and

WHEREAS, for ten dollars (\$10.00), and other good and valuable consideration enumerated in a written agreement dated 24 October 1996, the sufficiency of which is hereby acknowledged, HUL has agreed to share ownership of the aforementioned inventions improvements, applications, patents, reissues, extensions, and the like on a 50% / 50% equal basis with Ludwig Institute for Cancer Research, a Swiss not-for-profit corporation having an office at 1345 Avenue of the Americas, New York, New York 10105, United States of America (hereinafter LICR);

NOW, THEREFORE, HUL hereby assigns to LICR a fifty percent (50%) interest in the patent applications identified in the following LIST OF PATENT PROPERTIES, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said patent applications, and in any reissue or extension thereof.

LIST OF PATENT PROPERTIES

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>
08/510,133	01/08/95	Receptor Ligand
08/585,895	12/01/96	Receptor Ligand
08/601,132	14/02/96	Receptor Ligand
08/671,573	28/06/96	Receptor Ligand VEGF-C
PCT/FI96/00427	01/08/96	Receptor Ligand VEGF-C
08/795,430	02/05/97	Vascular Endothelial Growth Factor C (VEGF-C) Protein and Gene, Mutants Thereof, and Uses Thereof

WITNESS my hand this 25 day of April, Nineteen Hundred and Ninety-Seven.

Witnesses:

1) [Signature]
Name:

2) [Signature]
Name:

Helsinki University
Licensing, Ltd.

By: [Signature]
Heikki Lampi
President



PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	Title: RECEPTOR LIGAND
)	
Alitalo et al.)	
)	
Serial No. 08/510,133)	Art Unit: 1801
)	
Filed: August 1, 1995)	Examiner: Lathrop, B.
)	
)	

Change of Inventor's Address

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please be advised that the residence and mailing address of co-inventor Vladimir Joukov is now as follows:

51 Massachusetts Avenue, Apt. 1F
Boston, Massachusetts 02115

This notification is NOT intended as a change of correspondence address. Please continue to send correspondence to the Applicants' attorney at the address below:

Respectfully submitted,
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:



David A. Gass
Registration No. 38,153

Date: Feb 24, 1998



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/510,133	08/01/95	ALITALIA	08113/32843

HM11/0403
MARSHALL O'TOOLE GERSTEIN MURRAY
AND BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

EXAMINER	
HALLON S	
ART UNIT	PAPER NUMBER
1646	23

DATE MAILED:

04/03/98

Please find below a communication from the EXAMINER in charge of this application.

Commissioner of Patents

IN RESPONSE TO APPLICANT'S LETTER OF STATUS INQUIRY,
FILED 12/24/97, SEE ATTACHED.

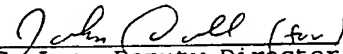


UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

In re Application of
KARI ALITALO, ET AL.
Serial No. 08/510,133
Filed: August 1, 1995
For: RECEPTOR LIGAND

:
:
: SUSPENSION
: OF PROSECUTION
:

A reference relevant to the examination of this application will soon become available. Ex parte prosecution is SUSPENDED pending the availability of the reference. Applicants will be notified when the reference becomes available and prosecution will resume at that time.

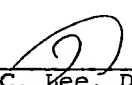

Mary C. Lee, Deputy Director
Patent Examining Group 1800

MARSHALL O'TOOLE GERSTEIN MURRAY
AND BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO, IL 60606-6402

In re Application of
KARI ALITALO, ET AL.
Serial No. 08/510,133
Filed: August 1, 1995
For: RECEPTOR LIGAND

:
:
: SUSPENSION
: OF PROSECUTION
:

A reference relevant to the examination of this application will soon become available. Ex parte prosecution is SUSPENDED pending the availability of the reference. Applicants will be notified when the reference becomes available and prosecution will resume at that time.



Mary C. Lee, Deputy Director
Patent Examining Group 1800


MARSHALL O'TOOLE GERSTEIN MURRAY
AND BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO, IL 60606-6402



Attorney Docket No. 28967/32863

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Kari Alitalo)	I hereby certify that this paper and the
)	documents referred to as enclosed
and Vladimir Joukov)	herewith are being deposited with the
)	United States Postal Service as First
Serial No.: 08/510,133)	Class Mail, postage prepaid, in an
)	envelope addressed to: Assistant
Filed: August 1, 1995)	Commissioner for Patents,
)	Washington, DC 20231, on this date:
For: RECEPTOR LIGAND)	
)	October 26, 1999
Group Art Unit: 1646)	
)	
Examiner: Saoud, C.)	
)	David A. Gass
)	Reg. No.: 38,153
)	Attorney for Applicants

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith are a Form PTO-1449 listing several documents, together with a copy of each listed document. The Applicants respectfully request that these documents be made of record and considered by the Examiner in the above-identified application.

Documents A1-A4 are U.S. priority documents of published PCT applications that are now publically available from WIPO.

Documents B8-B10, C112, C114, and C149-C152 were identified by the European Patent Office in an International Search Report for a related PCT application. A copy of the search report is also attached hereto.

Documents C115-C148 pertain to sequences, such as EST's, that have been posted in the Genbank Database, where the sequences should be available in computer readable form.

This Supplemental Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

The Commissioner is authorized to charge any fee required by this paper to Deposit Account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

October 26, 1999

By:



David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

* 1500000
SHEET 1 of 5

FILE COPY

Form PTO-1449 (Modified) U.S. Department of Commerce
Patent and Trademark Office

Oct 28 1999

INFORMATION DISCLOSURE STATEMENT

(Use several sheets if necessary)

Atty. Docket No. 28967/32863	Serial No. 08/510,133
Applicant Alitalo, K. et al.	
Filing Date August 1, 1995	Group 1646

U.S. PATENT DOCUMENTS							
*Examiner Initials		Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate
<i>CA</i>	A1	08/207,550	none	Jing-Shan Hu <i>et al.</i>	—	—	03/08/94
<i>CA</i>	A2	08/465,968	none	Crain Rosen <i>et al.</i>	—	—	06/06/95
<i>CA</i>	A3	60/003,491	none	James Lee <i>et al.</i>	—	—	09/08/95
<i>CA</i>	A4	08/554,374	none	Lyman, S.	—	—	11/08/95
<i>CA</i>	A5	5,326,695	07/05/94	Andersson <i>et al.</i>	435	70.1	
<i>CA</i>	A6	5,932,540	08/03/99	Jing-Shan Hu <i>et al.</i>	514	2	
<i>CA</i>	A7	5,935,820	08/10/99	Jing-Shan Hu <i>et al.</i>	435	69.4	

FOREIGN PATENT DOCUMENTS								
*Examiner Initials		Document Number	Publication Date	Country	Class	Subclass	Translation	
							Yes	No
<i>CA</i>	B7	0 506 477 A1	03/27/92	EP	—	—		
<i>CA</i>	B8	97/05250 A	02/13/97	WO	—	—		
<i>CA</i>	B9	97/09427 A	03/13/97	WO	—	—		
<i>CA</i>	B10	97/17442 A	05/15/97	WO	—	—		

EXAMINER <i>C. Saoud</i>	DATE CONSIDERED <i>4/12/00</i>
<p>*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.</p>	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date August 1, 1995	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
CA	C112	Achen, M.G. <i>et al.</i> , "Vascular Endothelial Growth Factor D (VEGF-D) is a Ligand for the Tyrosine Kinases VEGF Receptor 2 (Flk1) and VEGF Receptor 3 (Flt4)," <i>Proceedings of the National Academy of Science, USA</i> , 95:548-553 (January, 1998).
	C113	Adams, M.D. <i>et al.</i> , "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence," <i>Nature</i> , 377(6547 Supplement):3-174 (September, 1995).
	C114	Cohen, T. <i>et al.</i> , "VEGF121, A Vascular Endothelial Growth Factor (VEGF) Isoform Lacking Heparin Binding Ability, Requires Cell-Surface Heparan Sulfates for Efficient Binding to the VEGF Receptors of Human Melanoma Cells," <i>Journal of Biological Chemistry</i> , 270(19):11322-11326 (May 12, 1995).
	C115	Genbank AA151613, "z127h03.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 3'," Hillier, L. <i>et al.</i> , Dated 14-May-1997
	C116	Genbank AA425486, "zw46b06.r1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 5' similar to SW:VEGF_MOUSE Q00731 VASCULAR ENDOTHELIAL GROWTH FACTOR PRECURSOR," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997
	C117	Genbank N31713, "yy15b12.s1 Homo sapiens cDNA clone 271295 3'," Deposited by Hillier, L. <i>et al.</i> Dated 10-Jan-1996
	C118	Genbank N31720, "yy15d12.s1 Homo sapiens cDNA clone 271319 3'," Deposited by Hillier, L. <i>et al.</i> Dated 10-Jan-1996
	C119	Genbank AA406492, "zv12g06.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 75366 5'," Deposited by Hillier, L. <i>et al.</i> Dated 17-May-1997
	C120	Genbank N50972, "yy94b08.s1 Homo sapiens cDNA clone 281175 3'," Deposited by Hillier, L. <i>et al.</i> Dated 14-Feb-1996
CA	C121	Genbank AA421713, "zu24b03.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 738893 3'," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997

EXAMINER C. Saoud	DATE CONSIDERED 4/12/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date August 1, 1995	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
ca	C122	Genbank N94399, "zb76f04.s1 Soares senescent fibroblasts NbHSF Homo sapiens cDNA clone 309535 3'," Deposited by Hillier, L. <i>et al.</i> Dated 20-Aug-1996
	C123	Genbank H05177, "y185b08.r1 Homo sapiens cDNA clone 44993 5'," Deposited by Hillier, L. <i>et al.</i> Dated 21-Jun-1995
	C124	Genbank AA479987, "zv18h12.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 3'," Deposited by Hillier, L. <i>et al.</i> Dated 08-Aug-1997
	C125	Genbank H05134, " y185b08.s1 Homo sapiens cDNA clone 44993 3'," Deposited by Hillier, L. <i>et al.</i> Dated 21-Jun-1995
	C126	Genbank, AA298182 "EST113866 Bone VII Homo sapiens cDNA 5' end," Deposited by Adams, M.D. <i>et al.</i> Dated 18-Apr-1997
	C127	Genbank AA298283, "EST113896 Bone VII Homo sapiens cDNA 5' end similar to similar to vascular endothelial growth factor," Deposited by Adams, M.D. <i>et al.</i> Dated 18-Apr-1997
	C128	Genbank T81481, "yd29f07.s1 Homo sapiens cDNA clone 109669 3'," Deposited by Hillier, L. <i>et al.</i> Dated 15-Mar-1995
	C129	Genbank AA425303, "zw46b06.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 3', mRNA sequence," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997
	C130	Genbank Z40230, "H. sapiens partial cDNA sequence; clone c-1wf11," Deposited by Genexpress. Dated 21-Sep-1995
	C131	Genbank Z44272, "H. sapiens partial cDNA sequence; clone c-1wf11," Deposited by Genexpress. Dated 21-Sep-1995
	C132	Genbank AA478766, " zv18h12.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 5'," Deposited by Hillier, L. <i>et al.</i> Dated 08-Aug-1997
ca	C133	Genbank H96876, "yw04b12.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 3'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Nov-1996

EXAMINER C. Saoud	DATE CONSIDERED 4/12/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/32863	Serial No. 08/510,133
INFORMATION DISCLOSURE STATEMENT <i>(Use several sheets if necessary)</i>		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date August 1, 1995	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

OK	C134	Genbank H96533, "yw04b12.r1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Nov-1996
	C135	Genbank T81690, "yd29f07.r1 Homo sapiens cDNA clone 109669 5' similar to SP:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3," Deposited by Hillier, L. <i>et al.</i> Dated 15-Mar-1995
	C136	Genbank T84377, "yd37h08.r1 Homo sapiens cDNA clone 110463 5' similar to SP:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3," Deposited by Hillier, L. <i>et al.</i> Dated 16-Mar-1995
	C137	Genbank N42368, "yy15b11.r1 Homo sapiens cDNA clone 271293 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Jan-1996
	C138	Genbank N42374, "yy15d11.r1 Homo sapiens cDNA clone 271317 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Jan-1996
	C139	Genbank H81868, "yv83d09.s1 Homo sapiens cDNA clone 249329 3'," Deposited by Hillier, L. <i>et al.</i> Dated 09-Nov-1995
	C140	Genbank H81867, "yv83d09.r1 Homo sapiens cDNA clone 249329 5'," Deposited by Hillier, L. <i>et al.</i> Dated 09-Nov-1995
	C141	Genbank AA149461, "z127h03.r1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 5' similar to SW:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3 PRECURSOR," Deposited by Hillier, L. <i>et al.</i> Dated 14-May-1997
	C142	Genbank R77495, "yi79e04.s1 Homo sapiens cDNA clone 145470 3'," Deposited by Hillier, L. <i>et al.</i> Dated 07-Jun-1995
	C143	Genbank H07899, "y186g06.s1 Homo sapiens cDNA clone 45138 3'," Deposited by Hillier, L. <i>et al.</i> Dated 23-Jun-1995
	C144	Genbank T89295, "yd37h08.s1 Homo sapiens cDNA clone 110463 3'," Deposited by Hillier, L. <i>et al.</i> Dated 20-Mar-1995
OK	C145	Genbank C21512, "HUMGS0010510, Human Gene Signature, 3'-directed cDNA sequence," Deposited by Okubo, K. Dated 01-Oct-1996

EXAMINER C. Saoud	DATE CONSIDERED 4/12/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/510,133	08/01/95	ALITALO	K 28113/32863

HM12/0426
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

EXAMINER

SAOUD, C

ART UNIT	PAPER NUMBER
----------	--------------

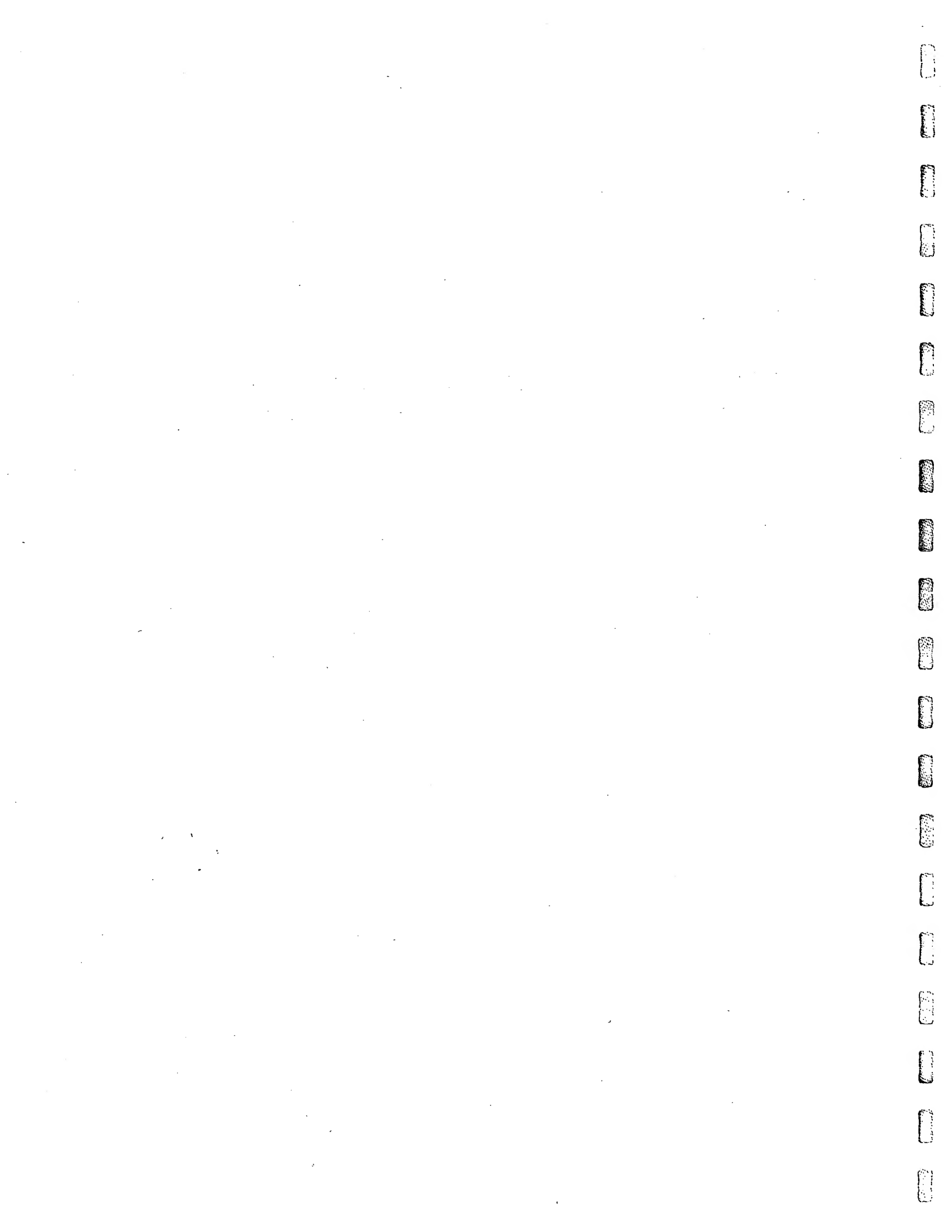
1646

26

DATE MAILED: 04/26/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks





UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/510,133	08/01/95	ALFONSO	28113/32563

FRANK S. DISIGLIO
SCULLY SCOTT MURPHY & PRESSER
400 GARDEN CITY PLAZA
GARDEN CITY NY 11530

H422/0629

EXAMINER

SAHID.C.

ART UNIT

PAPER NUMBER

1647

28

DATE MAILED:

06/29/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
081510.133			

EXAMINER

ART UNIT	PAPER NUMBER
----------	--------------

27

DATE MAILED:

INTERVIEW SUMMARY

All participants (applicant, applicant's representative, PTO personnel):

(1) Christine Saoud (3) DAVID GASS
(2) Gary Kunz (4) WILLIAM K. MERKEL
Date of Interview June 22, 2000 (5) FRANK S. DiCorglio

Type: ☐ Telephonic ☒ Personal (copy is given to ☐ applicant ☒ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No If yes, brief description: _____

Agreement ☐ was reached. ☒ was not reached.

Claim(s) discussed: 8, 9, 16, 17

Identification of prior art discussed: of record in case (Hu et al.)

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Discussed above clms in terms of prior art of record. Clm. 9 & 16 & 17 distinguished over the prior art of record. Additional limit to clm 8 of M.W. or C-terminal truncation would also distinguish over the prior art.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary. A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign this form unless it is an attachment to another form.

FORM PTOL-413 (REV.1-96)

Christine Saoud

Office Action Summary

Application No. 08/510,133	Applicant(s) ALITALO et al.
Examiner Christine Saoud	Group Art Unit 1646

- ☒ Responsive to communication(s) filed on Jun 11, 1997
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.
- A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

- ☒ Claim(s) 1, 2, 8, 9, 12-17, and 19-28 is/are pending in the application.
- Of the above, claim(s) _____ is/are withdrawn from consideration.
- ☐ Claim(s) _____ is/are allowed.
- ☒ Claim(s) 1, 2, 8, 9, 12-17, and 19-28 is/are rejected.
- ☐ Claim(s) _____ is/are objected to.
- ☐ Claims _____ are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been
- ☐ received.
- ☐ received in Application No. (Series Code/Serial Number) _____
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

- ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☐ Notice of References Cited, PTO-892
- ☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 25
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

227
147P

PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)
Serial No: 08/510,133)
Filed: August 1, 1995)
Title: Receptor Ligand)
Group Art Unit: 1646)
Examiner: Christine Saoud)

ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231


Sir:

The undersigned attorney of record in the above-identified application
hereby appoints as associate attorney(s):

Frank S. DiGiglio (Reg. No. 31,346)
Scully, Scott, Murphy & Presser
400 Garden City Plaza
Garden City, New York 11530
(516) 742-4343

to prosecute this application, to make alterations or amendments therein, and to
transact any and all business in the Patent and Trademark Office connected
therewith.

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN


David A. Gass
Registration No. 38,153

June 22, 2000



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):) Title: RECEPTOR LIGAND
Alitalo et al.)
Serial No: 08/510,133) Group Art Unit: 1646
Filed: August 1, 1995) Examiner: Christine Saoud

AMENDMENT TRANSMITTAL

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment for the above application.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on July 24, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

Verified statement(s) claiming small entity status is(are) attached.

- ☒ Small entity status has been established and is still effective.
Has not been established.

2. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	29	MINUS	23	= 6	X 9=	\$54.00	X18=	\$
INDEP.	4	MINUS	5	= 0	X39=	\$0	X78=	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim					+130=	\$	+260=	\$
TOTAL ADDITIONAL FEE						\$54.00	OR	\$

3. **Method of Payment of Fees**

- ☒ Attached is a check in the amount of: \$54.00
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

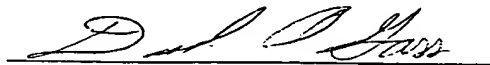
4. **Deposit Account and Refund Authorization**

- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.
- ☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:


David A. Gass
Reg. No: 38,153

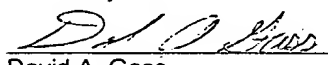
July 24, 2000



#29D 8/10/00
T. Bray

PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)	I hereby certify that this paper is being
Serial No: 08/510,133)	deposited with the United States Postal
Filed: August 1, 1995)	Service with sufficient postage as first
Title: Receptor Ligand)	class mail, postage prepaid, in an
Group Art Unit: 1646)	envelope addressed to: Assistant
Examiner: Christine Saoud)	Commissioner for Patents, Washington,
)	D.C., 20231 on this date:
)	
)	Date: July 24, 2000
)	
)	David A. Gass
)	Registration No. 38,153
)	Attorney for Applicants

AMENDMENT AND REPLY PURSUANT TO 37 C.F.R. §§ 1.111

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In an Office action mailed April 26, 2000, the Patent Office rejected claims 1, 2, 8, 9, 12-17, and 19-28 variously under 35 USC §102 and 112, first paragraph. The Applicants respectfully request reconsideration in light of the following amendments and remarks.

57/28/2000 VWRN11 00000055 118510133 54.00 DP
01 FC:203

AMENDMENTS

In the claims:

Please cancel all pending claims 1-2, 8-9, 12-17, and 19-28; and add new claims 29-57 as shown below:

¹
~~29.~~ A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of amino acids 1-180 of SEQ ID NO: 33 effective to permit such binding, said polypeptide lacking all of amino acids of SEQ ID NO: 33 beyond position 180.

²
~~30.~~ A pharmaceutical composition comprising a polypeptide according to claim ~~29~~ in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

³
~~31.~~ A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim ~~30~~.

⁴
~~32.~~ A polypeptide according to claim ~~29~~ further comprising a detectable label.

⁵
~~33.~~ A purified and isolated polypeptide according to claim ~~29~~ that binds Flt4 and stimulates Flt4 phosphorylation in mammalian cells expressing Flt4.

⁶
~~34.~~ A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim ~~29~~.

⁷
~~35.~~ A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase (Flt4), wherein the polypeptide comprises a portion of SEQ ID NO: 33 effective to permit such binding, and wherein the polypeptide has an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

⁸
36. A pharmaceutical composition comprising a polypeptide according to claim ⁷35 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

⁹
37. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim ⁸36.

¹⁰
38. A purified and isolated polypeptide according to claim ⁷35 that binds Flt4 and stimulates Flt4 phosphorylation in mammalian cells expressing Flt4.

¹¹
39. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express ¹⁰human Flt4 receptor tyrosine kinase with a polypeptide according to claim ¹⁰38.

¹²
40. A purified and isolated polypeptide according to claim ¹⁰38, said polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13.

¹³
41. A polypeptide according to claim ⁷35 further comprising a detectable label.

¹⁴
42. A polypeptide according to claim ⁷35 wherein said portion of SEQ ID NO: 33 effective to permit such binding is a continuous portion of SEQ ID NO: 33 within amino acids 1-180 of SEQ ID NO: 33.

¹⁵
43. A polypeptide according to claim ⁷35 wherein the amino terminus of said portion effective to permit such binding corresponds with position 34 of SEQ ID NO: 33.

¹⁶
44. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express ⁷human Flt4 receptor tyrosine kinase with a polypeptide according to claim ⁷35.

¹⁷
~~45~~. A purified and isolated polypeptide comprising a human polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, wherein amino terminal amino acids 2 through 18 of said human polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

¹⁸
~~46~~. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim ¹⁷~~45~~.

¹⁹
~~47~~. A purified and isolated polypeptide according to claim ¹⁷~~45~~ that binds Flt4 and stimulates Flt4 phosphorylation in mammalian cells expressing Flt4.

²⁰
~~48~~. A pharmaceutical composition comprising a polypeptide according to claim ¹⁷~~45~~ in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

²¹
~~49~~. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim ²⁰~~48~~.

²²
~~50~~. A polypeptide according to claim ¹⁷~~45~~ further comprising a detectable label.

²³
~~51~~. A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, wherein said polypeptide has an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions and is purifyable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

²⁴
~~52.~~ A polypeptide according to claim ²³~~51~~ which is capable of stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

²⁵
~~53.~~ A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim ²⁴~~52~~.

²⁶
~~54.~~ A polypeptide according to claim ²³~~51~~ further comprising a detectable label.

²⁷
~~55.~~ A pharmaceutical composition comprising a polypeptide according to claim ²³~~51~~ in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

²⁸
~~56.~~ A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim ²⁷~~55~~.

²⁹
~~57.~~ A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim ²³~~51~~.

REMARKS

I. Prosecution History.

The application as filed contained twelve claims. In a preliminary amendment (Paper No. 10) dated August 12, 1996, claims 1-4, 6, 8-9, and 12 were amended, and claims 13-19 were added to the application. In an amendment dated February 10, 1997, the applicants canceled claims 10 and 18, amended claims 1, 8, 9, 14, and 17, and added new claims 20-25. In an amendment dated June 11, 1997, the applicants canceled claims 3-7 and 11, amend claims 8, 16, 17, 21, 22, and 25, and add new claims 26-28. Thereafter, the Patent Office suspended prosecution for more than 2½ years on the grounds that a relevant reference "may

soon become available." After numerous written and telephonic status inquiries by the undersigned attorney, the Patent Office resumed prosecution and issued the outstanding Office Action.

In the present amendment, the Applicants cancel all pending claims;¹ and add new claims 29-57. Thus, upon entry of the foregoing amendments, claims 29-57 are pending. The following table correlates new and old claim numbers.

Current Claim	Previous Claim	Comments
29	8	Amended to clarify that claimed polypeptide does not include a large carboxy-terminal portion of SEQ ID NO: 33
30	12	
31	New	Method of using elected product
32	19	
33	14	
34	New	Method of using elected product
35	9	Amended to include limitations relating to high affinity binding and amino acid sequence (SEQ ID NO: 33)
36	12	
37	New	Method of using elected product
39	14	
39	New	Method of using elected product
40	15	
41	19	
42	26	
43	27	
44	New	Method of using elected product

¹ In an interview with the undersigned on June 22, 2000, the Examiner requested that the claims be presented in a renumbered claim set.

Current Claim	Previous Claim	Comments
45	16	Amended to recite "human"
46	New	Method of using elected product
47	14	
48	25	
49	New	Method of using elected product
50	28	
51	17 & 13	
52	25	
53	New	Method of using elected product
54	21	
55	22	
56	New	Method of using elected product
57	New	Method of using elected product

II. Explanation of amendments

Most of the amendments to the claims merely represent a consolidation of the claims for the purpose of conciseness and clarity. For example, words or phrases that have been added to claims have, for the most part, been taken from other claims that have been canceled. Many of the dependent claims are repetitive (but depend from different independent claims).

The amendment to independent claim 45 (formerly 16) to recite "human" finds support throughout the application, because Examples in the application describe the isolation and characterization of a human cDNA and protein.

The Applicants do not intend by these or any other amendments to abandon the subject matter or any claim as originally filed or later presented, and reserve the right to pursue such subject matter in continuing applications.

New claims 30, 32, 33, and 47 are dependent claims analogous to other dependent claims that had been pending in the application. (See Table.) Claims 31, 34, 37, 39, 44, 46, 49, 53, 56, and 57 are dependent method claims which the Applicants request to have favorably considered with their parent polypeptide and composition claims, upon allowance of those claims, consistent with the Patent Office's policy announced at 1184 OG 86 (March 26, 1996). Support for the dependent process claims is found throughout the application, including at pages 6-7.

III. The Patent Office's rejection of claims 1, 13-17, 19, 20-22, 23, 25, and 28 under 35 U.S.C. §112, first paragraph, for lack of written description should be withdrawn.

The Patent Office rejected claims 1, 13-17, 19, 20-22, 23, 25, and 28 under 35 U.S.C. §112, first paragraph, alleging that these claims contain subject matter which was not described in the specification in a way that reasonably conveys to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Applicants respectfully traverse.

A. The rejection of claims 1, 14, 15, 19, and 23 has been rendered moot.

The scope of the rejection has been narrowed by the amendments set forth above. Specifically, the Applicants have canceled rejected claims 1 and 23.² Several of the other rejected claims (e.g., claims 14, 15, 19 -- now embodied in claims 33, 38, 40, 41, and 47-- have been amended to depend (directly or indirectly) from claims 29 and 35 (which are similar to claims 8 and 9), which the Patent Office had acknowledged contain sufficient limitations to satisfy the written description requirement). The rejection of claims 14, 15, and 19 should therefore be withdrawn.

² The Patent Office's main allegation in support of its rejection is that "The broadest claim only requires the polypeptide to be capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase. The additional limitations of the dependent claims fail to provide the structure which is required for this receptor binding or for receptor activation (an additional functional limitation)." (Office action at p. 3.) As explained above, claim 1 (the claim that required only binding) has been canceled without prejudice.

B. The rejection of claims 13, 16, 17, 20-22, 25, and 28 should be withdrawn.

Claims 45 and 51 (which correspond with rejected claims 16 and 17) are the two independent claims of the remaining claims that stand rejected (13, 16, 17, 20-22, 25, and 28). As set forth below, the rejection of these claims (and their dependent claims) also should be withdrawn.

As part of the basis for its rejection, the Patent Office expressed concern that the claims encompassed non-human polypeptides and anti-flt4 antibody peptides. While the Applicants believe they are entitled to claim both of these categories of polypeptides in their patent applications, these concerns are moot with respect to the rejected claims. Claim 45 (which replaces claim 16) recites "A purified and isolated polypeptide comprising a human polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13." The recitation "human" negates the Patent Office's allegation that the claim encompasses polypeptides from other species, and the recitation of the amino sequence in SEQ ID NO: 13 would reasonably be expected to exclude anti-Flt4 antibodies, because the sequence of SEQ ID NO: 13 was not obtained from an antibody.

Claim 51, which replaces claim 17, recites, "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, wherein said polypeptide has an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions and is purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase." The Applicants respectfully submit that non-human polypeptides and anti-Flt4 antibodies are not purifiable from the PC-3 cell line.

The Patent Office observed that, "In making a determination of whether the application complies with the written description requirement of 35

U.S.C. 112, first paragraph, it is necessary to understand what Applicant has possession of and what Applicant is claiming." (Office action at p. 3.) However, when performing this analysis, the Patent Office then focused on nucleic acids:

From the specification, it is clear that Applicant has possession of a nucleic acid molecule which encodes a protein which has the amino acid sequence of SEQ ID NO:33. This nucleic acid molecule has a nucleic acid sequence of SEQ ID NO:32 and is contained within plasmid pFLT4-L (ATCC deposit #97231).

(Id.)

This decision to focus on nucleic acids suggests a possible misapprehension of the application, and a misapplication of "nucleic acid reasoning" to a polypeptide invention.

A review of the application shows that the Applicants had possession of the polypeptides that they are claiming *before* having possession of the nucleic acid on which the Patent Office has focused. Examples 4-5 of the application teach that an Flt4 ligand was isolated and purified from the conditioned media from the PC-3 prostatic adenocarcinoma cell line. (Specification, pages 15-19.) An affinity purification procedure was described that employed the Flt4 extracellular domain, also described in the application. (See Examples 3-5.) The purified polypeptide had a molecular weight of approximately 23 KD (SDS-PAGE, reducing conditions) and stimulated Flt4 phosphorylation. (See specification, paragraph bridging pages 18-19.) The polypeptide was sufficiently pure to determine an amino-terminal sequence which is set forth in SEQ ID NO: 13. (See specification at page 19, first full paragraph.) The purpose of the written description requirement is to ensure that inventors are in possession of what they are claiming, and when one considers the teachings of the Applicants examples, summarized above, it is abundantly clear to the reader that the Applicant was in possession of what is being claimed herein (e.g., in claims 45 and 51).³

In the rejection, the Patent Office also examines whether the claims explicitly recite "limitations to provide function":

³ Of course, the Application goes on to describe many more features of the invention, including the isolation of a cDNA encoding the protein, deduced amino acid sequence, and other properties. The entirety of the application supports broad genus claims which the Applicants intend to pursue in this application or related applications.

The claims lack structural limitations (i.e. claims 1, 14, 17, 19, 20, and 21 recite no structure at all other than a polypeptide) to provide the function of encoding a polypeptide which binds to the Flt4 receptor tyrosine kinase. Some of the claims include a molecular weight, however, this is not sufficient for providing the required function and some of the claims recite a portion of SEQ ID NO:13, however, this amino acid sequence is still not sufficient for providing the receptor binding activity required by the claims.

[T]he instant application fails to provide a written description of the species or the genus which are encompassed by the instant claims except for the polypeptide of SEQ ID NO:33. The specification does not provide a complete structure of those polypeptides which bind to the Flt4 receptor tyrosine kinase with high affinity. The claims also fail to recite other relevant identifying characteristics (physical and/or chemical and/or functional characteristics coupled with a known or disclosed correlation between function and structure) sufficient to describe the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention.

(Office action at pp. 4-5.)

The Patent Office is reminded that 35 USC §112, *first paragraph*, sets forth minimum requirements for *the specification*, not for the claims. The purpose of patent claims is to particularly point out and distinctly claim the subject matter of the invention (§112, *second paragraph*), in a way that appraises the public of what is within the scope of the invention and what is not. The pending claims satisfy this requirement.⁴

For all of these reasons, the rejection for lack of written description should be withdrawn.

⁴ No paragraph of Section 112 requires claim limitations to provide function as suggested by the Examiner. This is aptly demonstrated by the facts that (1) the Patent Office issued patent claims to proteins prior to the revolution of recombinant DNA technology which provided sequencing capabilities; and (2) the Patent Office continues to issue countless claims to antibodies (which are proteins) that do not recite variable region amino acid sequence or otherwise characterize the antibody variable region, except in functional terms.

IV. The Patent Office's rejection of claims 1, 12-17, 19, 21-23, 25, and 28 under 35 U.S.C. §112, first paragraph, for lack of enabling disclosure should be withdrawn.

In paragraph 7 of the Office action, the Patent Office rejected claims 1, 12-17, 19, 21-23, 25, and 28 under 35 U.S.C. §112, first paragraph, alleging that "the specification, while being enabling for polypeptides comprising a contiguous portion of SEQ ID NO:33 which specifically bind to Flt4 receptor tyrosine kinase, does not reasonably provide enablement for any polypeptide that specifically binds to Flt4 receptor tyrosine kinase, or for those polypeptides that have a molecular weight of 23 kD and bind, or for those polypeptides which comprise amino acid sequence SEQ ID NO:13 and bind. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims." The Applicants respectfully traverse.

A. The rejection of claims 1, 12, 14-15, 19, and 23 has been rendered moot.

The scope of the rejection also has been narrowed by the amendments set forth above. Specifically, the Applicants have canceled rejected claims 1 and 23.⁵ The new claims which correspond to several of the other rejected claims (e.g., claims 12, 14, 15, 19) have been amended to depend (directly or indirectly) from claims 29 and 35 (which are analogous to claims 8 and 9, which the Patent Office had acknowledged to be enabled by the specification). The rejection of claims 12, 14, 15, and 19 should therefore be withdrawn.

B. The rejection of claims 13, 16, 17, 21-22, 25, and 28 should be withdrawn.

Claims 45 and 51 (which correspond with rejected claims 16 and 17) are the two independent claims of the remaining claims that stand rejected (13, 16,

⁵ The Patent Office's main allegation in support of its rejection is that "The broadest claim only requires the polypeptide to be capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase. The additional limitations of the dependent claims fail to provide the structure which is required for this receptor binding or for receptor activation (an additional functional limitation)." (Office action at p. 3.) As explained above, claim 1, the claim that required only binding, has been canceled without prejudice.

17, 20-22, 25, and 28). As set forth below, the rejection of these claims (and their dependent claims) also should be withdrawn.

As explained in detail in the arguments relating to written description, claim 45 recites "human" and claim 51 recites purifyable from a human cell line. Claim 45 also recites a partial amino acid sequence to further identify the human polypeptide. The claims recite that the polypeptides have Flt4 binding activity, and the specification demonstrates that Flt4 affinity chromatography can be used successfully to isolate the claimed polypeptides.

The Patent Office urges that "The claims must recite sufficient structural elements to provide the recited functions, and one of ordinary skill in the art would not reasonably expect any 23 kD protein or protein which comprises SEQ ID NO:13 to bind to Flt4." The Applicants dispute that §112, first paragraph, contains any requirement of this sort. As explained above, 35 USC §112, first paragraph, sets forth minimum requirements for *the specification*, not for the claims. The purpose of patent claims is to particularly point out and distinctly claim the subject matter of the invention (§112, second paragraph), in a way that apprises the public of what is within the scope of the invention and what is not. It is not questioned that the pending claims satisfy this requirement.

If one makes the inquiry under §112, first paragraph, of whether *the specification* is enabling for the full scope of the claims, the answer is clearly affirmative. The Patent Office's rejection is clearly focused on the "how to make" aspect of the enablement requirement, and the present application teaches those skilled in the art several methodologies to make the subject matter of the invention, commensurate with the scope of claims 45 and 51 and dependent claims. For example, the patent application teaches that one can use affinity purification procedures to isolate the claimed polypeptide from cell sources. (The application also enables the production of antibodies to the receptor ligand that can be used for affinity purification procedures.) The application also teaches a human polynucleotide sequence that one can use to make polypeptides of the invention using the entire breadth of recombinant technologies known in the art. Additionally, because the application teaches a cDNA sequence, it enables one of ordinary skill in the art to screen any human source (human cell lines, human biological samples,

etc.) for allelic variants and the like, using, e.g., conventional hybridization procedures. Assuming that one uses this polynucleotide screening-based approach to identify polynucleotides that potentially encode alternative human alleles, the application also teaches Flt4 binding and activity assays to confirm that any novel sequences that one obtains, encodes, and expresses will satisfy the functional limitations of the claims.

For all of these reasons, the claims in the application are commensurate in scope with the subject matter enabled by the specification, and the rejection under §112, first paragraph, for lack of enablement should be withdrawn.

V. The rejection of claims 1-2, 8-9, 12-17, and 19-28 under 35 USC §102(e) as being anticipated by Hu et al. was improper, and should be withdrawn.

The Patent Office rejected claims 1-2, 8-9, 12-17, and 19-28 under 35 USC §102(e), alleging that the subject matter of these claims was anticipated by Hu et al., U.S. Patent No. 5,932,540:

Hu et al. disclose a polypeptide, SEQ ID NO:2, which is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation of mammalian cells expressing Flt4 receptor tyrosine kinase (see claims 1-60). Therefore, the instant claims are anticipated by the prior art.

With regard to claims 19, 21, and 28 which include a detectable label, Hu et al. disclose the polypeptide linked to a detectable label at column 17, lines 60-65, thus meeting this limitation.

(Office Action at pp. 9-10.)

The Applicants respectfully traverse.

At the outset, the Applicants dispute the Patent Office's characterization of Hu et al., because Hu et al. neither discloses nor suggests that any polypeptide binds Flt4. In fact, Hu et al. makes no mention of the Flt4 receptor whatsoever.

The Applicants also dispute the Patent Office's implication that the scope or wording of *the claims* of the Hu et al. patent have any relevance to whether Hu et al. is anticipatory under §102(e). The Hu et al. application was filed on

December 24, 1997, more than two years after the filing date of the present application, and after the publication of a PCT application based on the present application (See WO 97/05250, published February 13, 1997), and after the publication of the present inventors own work in prominent scientific journals that would have come to the attention of Hu et al. (See, e.g., Joukov et al., "A Novel Vascular Endothelial Growth Factor, VEGF-C, Is a Ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) Receptor Tyrosine Kinases," *EMBO J.*, 15(2): 290-298 (1996); and Joukov et al., "Proteolytic Processing regulates receptor specificity and activity of VEGF-C," *EMBO J.*, 16(13): 3898-3911 (1997).) Still more of the inventors' work was published in 1997-1999, during the pendency of the Hu et al. application, when Hu et al. had the opportunity to amend their claims. The relevant inquiry under §102(e) is the inquiry of what was "described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent." This inquiry requires the Patent Office to ignore what was claimed in the Hu et al. patent, which may have been tainted by knowledge of the present invention, as explained above. The relevant inquiry must focus only on what was described in those Hu et al. priority applications that have a filing date that could have preceded the invention date of the applicants.⁶ See, e.g., *In re Benno*, 226 USPQ 683, 686 (Fed. Cir. 1985) ("The scope of a patent's claims determines what infringes the patent; it is no measure of what it discloses. A patent discloses only that which it describes....")

Moreover, the Patent Office has apparently ignored the axiom that anticipation of a claim under §102 can be found only if the prior art discloses every element of the claim. See, e.g., *In re King*, 801 F.2d 1324, 1326 (Fed. Cir. 1986). The claims (as pending and amended herein) recite features that are neither disclosed nor suggested by Hu et al., including the following features:

- (1) A polypeptide that binds Flt4, comprises a portion of the SEQ ID NO: 33 amino acid sequence, and comprises a molecular weight of

⁶ The Applicants reserve the right to dispute whether Hu et al. qualifies as a §102(e) reference, on the grounds that Hu et al. is not a patent granted on an application filed before the invention thereof by the applicant.

- approximately 23 kD (see, e.g., claim 35 and claims dependent therefrom);
- (2) A polypeptide comprising a specified amino terminus defined with respect to SEQ ID NO: 13 (see, e.g., claims 45 and claims dependent therefrom, and claim 43);
 - (3) A polypeptide that binds Flt4, and comprises a continuous portion of SEQ ID NO: 33, said portion consisting of a portion within amino acids 1-180 of SEQ ID NO: 33 (see, e.g., claims 29 and claims dependent therefrom and claim 42); and
 - (4) A polypeptide of about 23 kD that is purifyable from PC-3-conditioned medium using Flt4 affinity procedures, as recited in claim 51 and claims dependent therefrom;⁷

The Patent Office has not pointed to any description or suggestion in Hu et al. of any of these features, and in fact, Hu et al. neither describes nor suggests such features.

Because all of the claims recite features that are neither disclosed nor suggested by Hu et al., the rejection under §102(e) must be withdrawn.

⁷ It is clear from the Application (e.g., pp. 18-19 and Figure 7) that the polypeptide purifyable from PC3 medium is not prepro-VEGF-C, but rather, e.g., a 23 kD form having an amino-terminal sequence corresponding to XEETIKFAAAHYNTEILK.

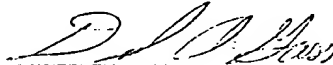
IV. Conclusion

The Applicants respectfully request entry of the foregoing amendments and allowance of all of the pending claims in view of the foregoing remarks.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

Dated: July 24, 2000


David A. Gass
Registration No. 38,153



GAU 1646
\$

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):) Title: RECEPTOR LIGAND
Alitalo et al.)
Serial No: 08/510,133) Group Art Unit: 1646
Filed: August 1, 1995) Examiner: Christine Saoud

AMENDMENT TRANSMITTAL

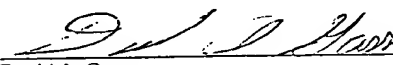
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment for the above application.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on July 24, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

RECEIVED

SEP 24 1995

WD

TECH CENTER 1600/2900 PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Alitalo et al.)	For: RECEPTOR LIGAND
Serial No. 08/510,133)	Art Unit: 1646
Filed: August 1, 1995)	Examiner: Saoud, C.

RECEIVED

RECEIVED

AMENDMENT AND STATEMENT PURSUANT TO 37 C.F.R. §1.825

Group 1600
Crystal Mall 1
7th Floor Reception Desk
Washington, D.C. 20231

F-130
1/13/00

Sir:

The Applicants request entry of the following amendment in the above-identified patent application prior to issuance of the next action on the merits.

1. **Small Entity Status**

Verified statement(s) claiming small entity status is(are) attached.

- ☒ Small entity status has been established and is still effective.
Has not been established.

2. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	29	MINUS	23	= 6	X 9=	\$54.00	X18=	\$
INDEP.	4	MINUS	5	= 0	X39=	\$0	X78=	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim					+130=	\$	+260=	\$
TOTAL ADDITIONAL FEE						\$54.00	OR	\$

3. **Method of Payment of Fees**

- ☒ Attached is a check in the amount of: \$54.00
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

4. **Deposit Account and Refund Authorization**

- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.
- ☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 

David A. Gass
Reg. No: 38,153

July 24, 2000

AMENDMENTS

Please amend the specification as follows:

At page 26, line 12, after "97231.", please insert --A 1997 base pair nucleotide sequence and the deduced amino acid sequence of the cDNA insert of this deposited plasmid is set forth in SEQ ID NOS: 34 and 35, respectively.--

Please delete pages 30-40 of the specification, which comprise the original Sequence Listing, and substitute therefor new pages 30-44, filed herewith, which constitute a substitute Sequence Listing. In view of this amendment, please renumber the pages of claims and abstract beginning with "45" (to preserve consecutive page numbering).

REMARKS

The present amendment modifies the application by introducing a 1997 bp DNA and deduced amino acid sequence corresponding to a Budapest treaty (ATCC) cDNA deposit that is referred to at page 26 and elsewhere in the application. The amendment to introduce these sequences will benefit the interested public by producing additional relevant information about the invention in the issued patent. None of the pending claims recites SEQ ID NOS: 34 or 35.

I hereby state that the content of the paper and computer-readable forms of the substitute Sequence Listing submitted herewith, for entry as part of the above-identified application, are the same as each other and do not introduce new matter into the disclosure of the application. All of the sequence information embodied in the substitute Sequence Listing filed herewith finds support in the application as originally filed, as explained below:

SEQ ID NOS: 1-33 of the original and substitute Sequence Listings are identical. Therefore, no new matter has been introduced in these sequences.


SEQ ID NOS: 34-35 of the substitute Sequence Listing depict a 1997 base pair nucleotide sequence and a deduced amino acid sequence, respectively, of a cDNA that was deposited with the ATCC and cross-referenced at p. 26 of the patent application as filed. These sequences are inherent properties of the deposited plasmid and thus find support in the deposited plasmid itself. See *Kennecott Corp. v. Kyocera International Inc.* 5 U.S.P.Q.2d 1194 (Fed. Cir. 1987) (The express description of an inherent property is not new matter and can be added to a specification with effect as of the original filing date); *In re Lundak*, 227 U.S.P.Q. 90 (Fed. Cir. 1985). The correlation between the sequences introduced by this

amendment and the ATCC deposit are further corroborated by the attached copies of two Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo that were filed for a related patent application, U.S. Serial No. 08/585,895. SEQ ID NOS: 34 and 35 of the substitute sequence listing for the present application (U.S. Serial No. 08/510,133) are identical to SEQ ID NOS: 44 and 45 of both Declarations. Paragraphs 4 & 5 of the Declaration filed November 26, 1997 and paragraphs 2-4 of the Declaration filed July 23, 1998 explain that complete sequencing of the cDNA insert that was deposited with the ATCC revealed a sequence that includes a 1997 base pair nucleotide sequence (SEQ ID NO: 34 of the substitute Sequence Listing field herewith) that encodes a 419 amino acid sequence (SEQ ID NO: 35 of the substitute Sequence Listing filed herewith). Thus, the present amendment is supported by the application as filed and does not introduce new matter. Entry of the amendment is respectfully requested.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, IL 60606-6402
Telephone: (312) 474-6300

By:


David A. Gass
Registration No. 38,153

August 22, 2000

EXHIBIT

PATENT
28967/33072

431

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996

Title: RECEPTOR LIGAND

Art Unit: 1646

Examiner: Saoud

) I hereby certify that this paper is being
) deposited with the United States Postal
) Service as first class mail, postage
) prepaid, in an envelope addressed to:
) Assistant Commissioner for Patents
) Washington, D.C. 20231, on this date:

) Dated: July 23, 1998

) 
) David A. Gass

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. KARI ALITALO

I, Kari Alitalo, do hereby declare and state as follows:

1. I am a co-inventor of the above-identified U.S. Patent Application (hereinafter "the patent application"). I am familiar with the Office action from the U.S. Patent and Trademark Office dated March 24, 1998, in the patent application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the issues and rejections raised in the Office action.

2. I understand that sequences identified as SEQ ID NOs: 44 and 45 were added to the patent application by an amendment dated November 26, 1997, and entered by the Patent Office on December 1, 1997. Copies of those two sequences are appended hereto. I understand that, at the time of the amendment, SEQ ID NOs: 44 and 45 were identified as a nucleotide sequence and a deduced amino acid sequence of a cDNA that was deposited with the American Type Culture Collection (ATCC) as plasmid pFLT4-L and that is cross referenced in the patent application at pages 28-29. I understand that the Patent Office has objected to the amendment to introduce these two sequences into the patent application on the

basis that such an amendment "introduces new matter into the disclosure." The Patent Office's basis for this allegation was as follows:

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97231 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO: 44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO: 44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "the approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO: 44."

(Office action dated March 24, 1998, at paragraph 10.)

3. I confirm that our laboratory sequenced the insert of the same plasmid that was designated pFLT4-L and that was deposited with the ATCC as ATCC Deposit No. 97231 and that is referred to at pages 28-29 of the patent application. The nucleotide sequence of the insert of this plasmid (ATCC Deposit No. 97231) includes the 1997 nucleotides of sequence set forth in SEQ ID NO: 44 as appended hereto and added to the patent application in the amendment dated November 26, 1997. The 419 residue amino acid sequence set forth in SEQ ID NO: 45 (as appended hereto and added to the patent application) is deduced from the sequence set forth in SEQ ID NO: 44.

4. The insert of plasmid pFLT4-L (ATCC Deposit No. 97231) contains additional (non-coding) sequence adjacent to the 1997 nucleotides of sequence set forth in SEQ ID NO: 44. The apparent size discrepancy between the approximately 2.1 kb size of the insert (as estimated by agarose gel electrophoresis analysis) and the 1997 nucleotides of sequence as set forth in SEQ ID NO: 44 is explained by the existence of this additional non-coding sequence in the plasmid insert.

Certification

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and

the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

July 17, 1998
Date

Kari Alitalo
Kari Alitalo

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1997 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
CCCCCCCCGC CTCTCCAAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCTCGCC      60
CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCGGTTT CCTGTGAGGC      120
TTTACCTGA CACCCGCCGC CTTCCCCGG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG      180
GGAACGCGGA GCCCCGGACC CGTCCCCGCC GCCTCCGGCT CGCCAGGGG GGGTCGCCGG      240
GAGGAGCCCG GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGG GCGCGCGCCC      300
CCACCCCTGC CCCC GCCAGC GGACCGTCC CCCACCCCG GTCCTTCAC C ATG CAC      357
                                         Met His
                                         1

TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG      405
Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu
                    5                    10                    15

CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC TTC GAG TCC      453
Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe Glu Ser
                20                25                30

GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT      501
Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala
                35                40                45                50

TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA      549
Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val
                    55                    60                    65

GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG      597
Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys
                    70                    75                    80

TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC      645
Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn
                    85                    90                    95
```

CTC	AAC	TCA	AGG	ACA	GAA	GAG	ACT	ATA	AAA	TTT	GCT	GCA	GCA	CAT	TAT	693
Leu	Asn	Ser	Arg	Thr	Glu	Glu	Thr	Ile	Lys	Phe	Ala	Ala	Ala	His	Tyr	
100						105					110					
AAT	ACA	GAG	ATC	TTG	AAA	AGT	ATT	GAT	AAT	GAG	TGG	AGA	AAG	ACT	CAA	741
Asn	Thr	Glu	Ile	Leu	Lys	Ser	Ile	Asp	Asn	Glu	Trp	Arg	Lys	Thr	Gln	
115					120					125					130	
TGC	ATG	CCA	CGG	GAG	GTG	TGT	ATA	GAT	GTG	GGG	AAG	GAG	TTT	GGA	GTC	789
Cys	Met	Pro	Arg	Glu	Val	Cys	Ile	Asp	Val	Gly	Lys	Glu	Phe	Gly	Val	
				135					140					145		
GCG	ACA	AAC	ACC	TTC	TTT	AAA	CCT	CCA	TGT	GTG	TCC	GTC	TAC	AGA	TGT	837
Ala	Thr	Asn	Thr	Phe	Phe	Lys	Pro	Pro	Cys	Val	Ser	Val	Tyr	Arg	Cys	
			150					155					160			
GGG	GGT	TGC	TGC	AAT	AGT	GAG	GGG	CTG	CAG	TGC	ATG	AAC	ACC	AGC	ACG	885
Gly	Gly	Cys	Cys	Asn	Ser	Glu	Gly	Leu	Gln	Cys	Met	Asn	Thr	Ser	Thr	
		165					170					175				
AGC	TAC	CTC	AGC	AAG	ACG	TTA	TTT	GAA	ATT	ACA	GTG	CCT	CTC	TCT	CAA	933
Ser	Tyr	Leu	Ser	Lys	Thr	Leu	Phe	Glu	Ile	Thr	Val	Pro	Leu	Ser	Gln	
	180					185					190					
GGC	CCC	AAA	CCA	GTA	ACA	ATC	AGT	TTT	GCC	AAT	CAC	ACT	TCC	TGC	CGA	981
Gly	Pro	Lys	Pro	Val	Thr	Ile	Ser	Phe	Ala	Asn	His	Thr	Ser	Cys	Arg	
195					200				205						210	
TGC	ATG	TCT	AAA	CTG	GAT	GTT	TAC	AGA	CAA	GTT	CAT	TCC	ATT	ATT	AGA	1029
Cys	Met	Ser	Lys	Leu	Asp	Val	Tyr	Arg	Gln	Val	His	Ser	Ile	Ile	Arg	
				215					220					225		
CGT	TCC	CTG	CCA	GCA	ACA	CTA	CCA	CAG	TGT	CAG	GCA	GCG	AAC	AAG	ACC	1077
Arg	Ser	Leu	Pro	Ala	Thr	Leu	Pro	Gln	Cys	Gln	Ala	Ala	Asn	Lys	Thr	
			230					235					240			
TGC	CCC	ACC	AAT	TAC	ATG	TGG	AAT	AAT	CAC	ATC	TGC	AGA	TGC	CTG	GCT	1125
Cys	Pro	Thr	Asn	Tyr	Met	Trp	Asn	Asn	His	Ile	Cys	Arg	Cys	Leu	Ala	
		245				250						255				
CAG	GAA	GAT	TTT	ATG	TTT	TCC	TCG	GAT	GCT	GGA	GAT	GAC	TCA	ACA	GAT	1173
Gln	Glu	Asp	Phe	Met	Phe	Ser	Ser	Asp	Ala	Gly	Asp	Asp	Ser	Thr	Asp	
	260				265					270						
GGA	TTC	CAT	GAC	ATC	TGT	GGA	CCA	AAC	AAG	GAG	CTG	GAT	GAA	GAG	ACC	1221
Gly	Phe	His	Asp	Ile	Cys	Gly	Pro	Asn	Lys	Glu	Leu	Asp	Glu	Glu	Thr	
	275				280					285					290	
TGT	CAG	TGT	GTC	TGC	AGA	GCG	GGG	CTT	CGG	CCT	GCC	AGC	TGT	GGA	CCC	1269
Cys	Gln	Cys	Val	Cys	Arg	Ala	Gly	Leu	Arg	Pro	Ala	Ser	Cys	Gly	Pro	
				295					300						305	
CAC	AAA	GAA	CTA	GAC	AGA	AAC	TCA	TGC	CAG	TGT	CTC	TCT	AAA	AAC	AAA	1317
His	Lys	Glu	Leu	Asp	Arg	Asn	Ser	Cys	Gln	Cys	Val	Cys	Lys	Asn	Lys	
			310					315					320			

CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr 325 330 335	1365
TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn 340 345 350	1413
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu 355 360 365 370	1461
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg 375 380 385	1509
CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser 390 395 400	1557
GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met 405 410 415	1605
AGC TAAGATTGTA CTGTTTTCCA GTTCATCGAT TTTCTATTAT GGAAAACTGT Ser	1658
GTTGCCACAG TAGAACTGTC TGTGAACAGA GAGACCCTTG TGGGTCCATG CTAACAAAGA	1718
CAAAAGTCTG TCTTTCCTGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGCTCATC	1778
TGCAAAAGGC CTCTTGTAAG GACTGGTTTT CTGCCAATGA CCAACAGGCC AAGATTTTCC	1838
TCTTGTGATT TCTTTAAAAG AATGACTATA TAATTTATTT CCACTAAAAA TATTGTTTCT	1898
GCATTCATTT TTATAGCAAC AACAATTGGT AAAACTCACT GTGATCAATA TTTTATATC	1958
ATGCAAAATA TGTTTAAAT AAAATGAAAA TTGTATTAT	1997

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 419 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
1 5 10 15
Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe
20 25 30

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
 35 40 45
 Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
 50 55 60
 Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
 65 70 75 80
 Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
 85 90 95
 Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
 100 105 110
 His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
 115 120 125
 Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
 130 135 140
 Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
 145 150 155 160
 Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
 165 170 175
 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
 180 185 190
 Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
 195 200 205
 Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
 210 215 220
 Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
 225 230 235 240
 Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
 245 250 255
 Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
 260 265 270
 Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
 275 280 285
 Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
 290 295 300
 Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
 305 310 315 320
 Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
 325 330 335

Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
 340 345 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
 355 360 365

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
 370 375 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
 385 390 395 400

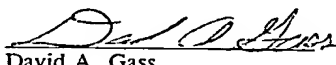
Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
 405 410 415

Gln Met Ser

EXHIBIT

PATENT
28967/33072

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

In re Application of:)	I hereby certify that this paper is being
Alitalo et al.)	deposited with the United States Postal
Serial No.: 08/585,895)	Service as first class mail, postage
Filed: January 12, 1996)	prepaid, in an envelope addressed to:
Title: RECEPTOR LIGAND)	Assistant Commissioner for Patents
Art Unit: 1801)	Washington, D.C. 20231, on this date:
Examiner: Lathrop, B.)	Dated: <u>Nov 26, 1997</u>
)	
)	David A. Gass
)	Registration No. 38,153

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. KARI ALITALO

1. I am a co-inventor of the above-identified U.S. Patent Application (hereinafter "the patent application"). I am familiar with the Office action from the U.S. Patent and Trademark Office dated May 28, 1997, in the patent application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the issues and rejections raised in the Office action.

Isolation of VEGF-C protein and cDNA

2. The present invention relates generally to a protein ligand for Flt4 receptor tyrosine kinase (VEGFR-3), which our research team has designated "VEGF-C." As taught in Example 14 of the patent application, VEGF-C also stimulates KDR/Flk-1 receptor tyrosine kinase (VEGFR-2). Our research team purified a VEGF-C protein that we discovered in conditioned media from a PC-3 prostatic adenocarcinoma cell line. We demonstrated that this protein bound to the extracellular domain of Flt4 and stimulated Flt4 phosphorylation. (See the patent application at Examples 4-5, for example.) Using SDS polyacrylamide gel electrophoresis, the VEGF-C protein was originally determined to have a molecular weight of about 23 kilodaltons. This measurement is in good

agreement with subsequent measurements of VEGF-C that we have recombinantly expressed in multiple cell lines, where we have determined the molecular weight to be about 21-23 kD.)

3. We sequenced the amino terminus of this purified VEGF-C protein as taught in the patent application in Example 5. (See especially p. 23.) I hereby reaffirm that our sequencing data from this protein is correctly reported in the patent application at p. 23 and in SEQ ID NO: 13.

4. As taught in Examples 6-10 of the patent application, we used the amino terminal amino acid sequence taught in the patent application to obtain a cDNA encoding VEGF-C. A plasmid containing the cDNA that is described in Example 11 of the patent application was deposited with the American Type Culture Collection and accorded ATCC accession number 97231.

5. The patent application describes a partial nucleotide sequence and a 350 amino acid open reading frame of the deposited VEGF-C cDNA. (See SEQ ID NOs: 32 and 33 of the patent application.) In the amendment filed herewith, these sequences have been amended such that the designation of residue "1" therein corresponds with the first residue of VEGF-C purified from PC-3 conditioned medium as described in the patent application. (See also paragraph 3, above.) Amended SEQ ID NOs: 32-33 are attached hereto as Exhibit A. Complete sequencing of the cDNA subsequently demonstrated that the translated open reading frame is actually 419 amino acids: it extends 69 codons upstream of what is reported in SEQ ID NO: 33. Attached hereto as Exhibit B is a 1997 nucleotide sequence of the cDNA that was deposited with the ATCC. Exhibit B also depicts the deduced 419 amino acid open reading frame. These sequences have been added to the patent application as SEQ ID NOs: 44 and 45. I shall use the term "prepro-VEGF-C" herein to refer to a polypeptide consisting of this 419 amino acid sequence.

6. As taught in the patent application (e.g., at p. 11), the carboxyl-terminal amino acid sequences encoded by the VEGF-C cDNA show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring 3 protein (BR3P) sequence that was

known in the art. (See Dignam and Case, *Gene*, 88:133-40 (1990); and Paulsson, *et al.*, *J. Mol. Biol.*, 211:331-49 (1990), both of record and cited in the patent application). The distinctive BR3P cysteine motifs (Cys-Xaa_n-Cys-Xaa-Cys-Xaa-Cys, wherein Xaa is any residue and n is variable) occur at least four times in the carboxy-terminal portion of VEGF-C (see Cys residues in Exhibit B at positions 280, 291, 293, and 295; positions 304, 315, 317, and 319; positions 328, 339, 341, and 343; and positions 347, 358, 360, and 362).

**VEGF-C processing and determination of
VEGF-C fragments that bind to Flt4.**

7. The Patent application teaches that the protein encoded by the VEGF-C gene is proteolytically processed, and teaches procedures to characterize this processing, such as analysis using antibodies and pulse-chase experiments. The application further teaches to screen truncated forms of VEGF-C (e.g., deletion fragments) to determine the portions of VEGF-C that are necessary to bind and stimulate Flt4. (See, e.g., pp. 29- 30 of the patent application.) Using techniques such as those described at pp. 29-30 of the patent application and mutational analysis, our research team has extensively characterized the processing of human prepro-VEGF-C in mammalian cell lines.

A. Our results from pulse-chase experiments indicate that the apparent first proteolytic processing of human prepro-VEGF-C involves cleavage of a signal peptide of about 31 residues, leaving residues 32-419 (hereinafter "pro-VEGF-C"). Pro-VEGF-C has an apparent molecular weight of about 55-58 kD.

B. We next observed that pro-VEGF-C is cleaved, either intracellularly or at the cell surface, into polypeptides of about 29 kD and about 31-32 kD (when assessed by SDS-PAGE under reducing conditions). The ~32 kD polypeptide binds the extracellular domain of Flt4 receptor tyrosine kinase with high affinity. (See Example 13 of the patent application.) The ~32 kD polypeptide was purified with immunoaffinity chromatography using an anti-VEGF-C antibody. The amino-terminus of

this purified polypeptide was determined to correspond to position 32 of the sequence shown in Exhibit B. Thus, the ~ 32 kD polypeptide represents the amino-terminal product of this proteolytic cleavage. Sequencing of the ~ 29 kD polypeptide indicated that cleavage occurred after amino acid 227 of the 419 amino acid sequence depicted in Exhibit B. (Amino acid 227 corresponds to residue 125 of SEQ ID NO: 33 in the patent application (Exhibit A).) This carboxy-terminal fragment of about 29 kD presumably includes residues 228-419 of the sequence depicted in Exhibit B (residues 126-317 of SEQ ID NO: 33). Thus, the ~ 29 kD polypeptide includes all of the Balbiani ring 3 protein cysteine motifs of VEGF-C (see paragraph 6 above). These results indicate that polypeptide fragments of the sequences depicted in Exhibits A or B that lack any domain having cysteine motifs of a Balbiani ring 3 protein (e.g., that lack the ~ 29 kD carboxy-terminal fragment) remain capable of binding with the extracellular domain of Flt4.

C. We also have observed forms of VEGF-C that reflect further proteolytic processing at the amino terminus. For the purpose of this declaration, I shall collectively refer to forms of VEGF described below as "mature VEGF-C."

- i. As indicated in paragraph 3, above, VEGF-C isolated from conditioned medium of PC-3 cells has an amino terminus corresponding to amino acid 103 in Exhibit B (i.e., amino acid 1 of SEQ ID NO: 33 (Exhibit A)).
- ii. We have sequenced VEGF-C that was recombinantly expressed in 293-EBNA cells (as described in Example 11 of the patent application) and determined that the amino terminus of this form corresponds with position 112 of the sequence shown in Exhibit B (i.e., position 10 of SEQ ID NO: 33 (Exhibit A)).

8. Our research team modified the human VEGF-C cDNA to recombinantly produce a fragment consisting of amino acids 104-213 of the 419 amino acid polypeptide in yeast (i.e., residues 2-111 of SEQ ID NO: 33). This fragment was shown to bind Flt4 and stimulate phosphorylation of both Flt4 (VEGFR-3) and KDR (VEGFR-2). In another experiment, a fragment lacking residues 1-112 of the 419 amino acid polypeptide retained receptor binding activity.

9. Collectively, the experimental results described in the preceding paragraphs indicate that polypeptides lacking amino acids 1-112 and 214-419 of the 419 residue amino acid sequence shown in Exhibit B retain Flt4 binding and stimulating activities. Stated differently, we have experimental evidence to indicate that a polypeptide corresponding to positions 11-112 of SEQ ID NO: 33 will retain Flt4 binding and stimulating activities. Moreover, one skilled in the art understands from the patent application how to perform receptor binding and phosphorylation assays, to localize further the portion of SEQ ID NO: 33 that is required for activity.

**The application enables one to obtain
VEGF-C-encoding cDNAs from non-human sources**

10. I infer from page 5 of the Office action that the Patent Office has rejected a claim of the application in part because of the lack of a claim limitation with respect to the source animal for VEGF-C. This section of the declaration provides evidence that the teachings in the patent application of a human VEGF-C cDNA, combined with the teachings that VEGF-C protein binds Flt4 (VEGFR-3) and VEGFR-2, enable one to obtain VEGF-C-encoding cDNAs from non-human sources.

11. To clone a murine VEGF-C cDNA, approximately 1×10^6 bacteriophage lambda clones of a commercially-available 12 day mouse embryonal cDNA library (lambda EXlox library, Novagen, catalog number 69632-1) were screened with a radiolabeled fragment of human VEGF-C cDNA containing nucleotides 495 to 1661 of the nucleotide sequence shown in Exhibit B. One positive clone was isolated.

12. A 1323 bp *EcoRI/HindIII* fragment of the insert of the isolated mouse cDNA clone was subcloned into the corresponding sites of the pBluescript SK+ vector (Stratagene) and sequenced. The cDNA sequence of this clone was homologous to the human VEGF-C sequence reported herein, except that about 710 bp of 5'-end sequence present in the human clone was not present in the mouse clone.

13. For further screening of mouse cDNA libraries, a *HindIII-BstXI* (*HindIII* site is from the pBluescript SK+ polylinker) fragment of 881 bp from the coding region of the mouse cDNA clone was radiolabeled and used as a probe to screen two additional mouse cDNA libraries. Two additional cDNA clones from an adult mouse heart ZAP II cDNA library (Stratagene, catalog number 936306) were identified. Three additional clones also were isolated from a mouse heart 5'-stretch-plus cDNA library in λ gt11 (Clontech Laboratories, Inc., catalog number ML5002b). Of the latter three clones, one was found to contain an insert of about 1.9 kb. The insert of this cDNA clone was subcloned into *EcoRI* sites of pBluescript SK+ vector and both strands of this clone were completely sequenced, resulting in the nucleotide and deduced amino acid sequences shown in Exhibit C. It is expected that the mouse VEGF-C polypeptide depicted in Exhibit C is processed into a mature mouse VEGF-C protein, in a manner analogous to the processing of the human prepro-VEGF-C.

14. The foregoing results demonstrate the utility of human VEGF-C-encoding polynucleotides of the invention for identifying and isolating polynucleotides encoding other non-human mammalian VEGF-C proteins. Such identified and isolated polynucleotides, in turn, can be expressed (using procedures similar to those described in the patent application for human VEGF-C) to produce recombinant polypeptides corresponding to non-human mammalian forms of VEGF-C.

15. The identity of the mouse protein as VEGF-C was confirmed by recombinantly expressing the above-described mouse cDNA, and analyzing the expressed proteins.

A. The 1.8 kb mouse VEGF-C cDNA was cloned as an *EcoRI* fragment into the retroviral expression vector pBabe-puro containing the SV40 early promoter region [Morgenstern *et al.*, *Nucl. Acids Res.*, 18:3587-3595 (1990)], and transfected into the Bosc23 packaging cell line [Pearet *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 90:8392-8396 (1994)] by the calcium-phosphate precipitation method. For comparison, Bosc23 cells also were transfected with the previously-described human VEGF-C construct in the pREP7 expression vector. The expressed proteins were immunoprecipitated with polyclonal antibodies raised against mature human VEGF-C.

B. Immunoprecipitation of VEGF-C from media of transfected and metabolically-labelled cells revealed bands of approximately $30\text{-}32 \times 10^3 M_r$ (a doublet) and $22\text{-}23 \times 10^3 M_r$ in 12.5% SDS-PAGE. These bands were not detected in samples from nontransfected or mock-transfected cells. These results demonstrate that antibodies raised against human VEGF-C recognize the corresponding mouse protein.

C. For receptor binding experiments, 1 ml aliquots of media from metabolically-labelled Bosc23 cells were incubated with VEGFR-3 extracellular domain, covalently coupled to sepharose, for 4 hours at 4°C with gentle mixing. (See Examples 4 and 5 in the patent application.) The sepharose beads were washed four times with ice-cold phosphate buffered saline (PBS), and the samples were analyzed by gel electrophoresis as described in Joukov *et al.*, *EMBO J.*, 15:290-298 (1996).

D. Similar $30\text{-}32 \times 10^3 M_r$ doublet and $22\text{-}23 \times 10^3 M_r$ polypeptide bands were obtained in the receptor binding assay as compared to the immunoprecipitation assay. In additional experiments, mouse VEGF-C appeared to be a potent inducer of VEGFR-3 autophosphorylation, too. Thus, the putative mouse VEGF-C binds and stimulates human VEGFR-3, confirming its identity. The slightly faster mobility of the mouse VEGF-C polypeptides that was observed may be caused by the four amino acid

residue difference observed in sequence analysis (residues H88-E91).
Murine VEGF-C appeared to bind VEGFR-2 with lower affinity.

16. The human VEGF-C cDNA also was used to design probes for successfully isolating a quail VEGF-C cDNA from a quail cDNA library. A fragment of the human VEGF-C cDNA comprising nucleotides 495-1661 of Exhibit B was obtained by PCR amplification, cloned into the pCRII vector (Invitrogen) according to the manufacturer's instructions, and amplified. The insert was isolated by *Eco* RI digestion and preparative gel electrophoresis and then labelled using radioactive dCTP and random priming. A cDNA library made from quail embryos of stage E-4 in pcDNA-1 vector (Invitrogen) was then screened using this probe. About 200,000 colonies were plated and filter replicas were hybridized with the radioactive probe under reduced stringency conditions (washes at 42°C with a wash solution comprising 2x SSC/0.1% SDS). Nine positive clones were identified and secondarily plated. Two of the nine clones hybridized in secondary screening. The purified clones (clones 1 and 14) had approximately 2.7 kb *Eco* RI inserts. Both clones were amplified and then sequenced using the T7 and SP6 primers (annealing to the vector). In addition, an internal *Sph* I restriction endonuclease cleavage site was identified about 1.9 kb from the T7 primer side of the vector and used for subcloning 5'- and 3'- *Sph* I fragments, followed by sequencing from the *Sph* I end of the subclones. The sequences obtained were identical from both clones and showed a high degree of similarity to the human VEGF-C coding region. Subsequently, walking primers were made in both directions and double-stranded sequencing was completed for 1743 base pairs, including the full-length open reading frame.

17. The cDNA sequence obtained includes a long open reading frame and 5' untranslated region. The DNA and deduced amino acid sequences for the quail cDNA are set forth in Exhibit D. Studies performed with the putative quail VEGF-C cDNA have shown that its protein product is secreted from transfected cells and interacts with avian VEGFR-3 and VEGFR-2, further confirming the conclusion that the cDNA encodes a quail VEGF-C protein.

18. As shown in Exhibit E, the human, murine, and avian (quail) VEGF-C precursor amino acid sequences share a significant degree of conservation. This high degree of homology confirms the likelihood of success of attempts to isolate VEGF-C encoding sequences from other species, especially vertebrate species, and more particularly mammalian and avian species, using human VEGF-C-encoding polynucleotides taught in the patent application as probes and using standard molecular biological techniques. The identity of putative VEGF-C-encoding cDNAs is confirmed using receptor binding studies such as the studies described in the patent application.

Certification

19. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

November 20, 1997
Date

Jan Alitalo
Kari Alitalo

EXHIBIT A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAAGTC	ATG	ACT	GTA	CTC	TAC	CCA	54
	Met	Thr	Val	Leu	Tyr	Pro	
	-33			-30			
GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA							102
Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln							
	-25					-15	
CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA							150
His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile							
	-10			-5		1	5
AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT							198
Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp							
			10			15	20
AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT							246
Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp							
			25			30	35
GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA							294
Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro							
			40			45	50
TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG							342
Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu							
			55			60	65
CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA							390
Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu							
			70			75	80
ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT							438
Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe							
			90			95	100
GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA							486
Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg							
			105			110	115
CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG							534
Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln							
			120			125	130
TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT							582
Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn							
			135			140	145
CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT							630
His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp							
			150			155	160
GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC							678
Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn							
			170			175	180
AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT							726
Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu							
			185			190	195
CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC							774
Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys							
			200			205	210

200										205										210										
CAG	TGT	GTC	TGT	AAA	AAC	AAA	CTC	TTC	CCC	AGC	CAA	TGT	GGG	GCC	AAC	822														
Gln	Cys	Val	Cys	Lys	Asn	Lys	Leu	Phe	Pro	Ser	Gln	Cys	Gly	Ala	Asn															
215						220					225																			
CGA	GAA	TTT	GAT	GAA	AAC	ACA	TGC	CAG	TGT	GTA	TGT	AAA	AGA	ACC	TGC	870														
Arg	Glu	Phe	Asp	Glu	Asn	Thr	Cys	Gln	Cys	Val	Cys	Lys	Arg	Thr	Cys															
230					235					240					245															
CCC	AGA	AAT	CAA	CCC	CTA	AAT	CCT	GGA	AAA	TGT	GCC	TGT	GAA	TGT	ACA	918														
Pro	Arg	Asn	Gln	Pro	Leu	Asn	Pro	Gly	Lys	Cys	Ala	Cys	Glu	Cys	Thr															
				250				255					260																	
GAA	AGT	CCA	CAG	AAA	TGC	TTG	TTA	AAA	GGA	AAG	AAG	TTC	CAC	CAC	CAA	966														
Glu	Ser	Pro	Gln	Lys	Cys	Leu	Leu	Lys	Gly	Lys	Lys	Phe	His	His	Gln															
			265				270						275																	
ACA	TGC	AGC	TGT	TAC	AGA	CGG	CCA	TGT	ACG	AAC	CGC	CAG	AAG	GCT	TGT	1014														
Thr	Cys	Ser	Cys	Tyr	Arg	Arg	Pro	Cys	Thr	Asn	Arg	Gln	Lys	Ala	Cys															
		280				285						290																		
GAG	CCA	GGA	TTT	TCA	TAT	AGT	GAA	GAA	GTG	TGT	CGT	TGT	GTC	CCT	TCA	1062														
Glu	Pro	Gly	Phe	Ser	Tyr	Ser	Glu	Glu	Val	Cys	Arg	Cys	Val	Pro	Ser															
		295				300					305																			
TAT	TGG	AAA	AGA	CCA	CAA	ATG	AGC	TAAGATTGTA	CTGTTTTC	CA	GTTCATCGAT					1116														
Tyr	Trp	Lys	Arg	Pro	Gln	Met	Ser																							
310					315																									
TTTCTATTAT	GGAAACTGT	GTTC														1140														

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 350 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met	Thr	Val	Leu	Tyr	Pro	Glu	Tyr	Trp	Lys	Met	Tyr	Lys	Cys	Gln	Leu
-33			-30					-25					-20		
Arg	Lys	Gly	Gly	Trp	Gln	His	Asn	Arg	Glu	Gln	Ala	Asn	Leu	Asn	Ser
		-15					-10					-5			
Arg	Thr	Glu	Glu	Thr	Ile	Lys	Phe	Ala	Ala	Ala	His	Tyr	Asn	Thr	Glu
	1				5					10					15
Ile	Leu	Lys	Ser	Ile	Asp	Asn	Glu	Trp	Arg	Lys	Thr	Gln	Cys	Met	Pro
			20					25						30	
Arg	Glu	Val	Cys	Ile	Asp	Val	Gly	Lys	Glu	Phe	Gly	Val	Ala	Thr	Asn
		35					40					45			
Thr	Phe	Phe	Lys	Pro	Pro	Cys	Val	Ser	Val	Tyr	Arg	Cys	Gly	Gly	Cys
		50				55						60			
Cys	Asn	Ser	Glu	Gly	Leu	Gln	Cys	Met	Asn	Thr	Ser	Thr	Ser	Tyr	Leu
	65				70						75				
Ser	Lys	Thr	Leu	Phe	Glu	Ile	Thr	Val	Pro	Leu	Ser	Gln	Gly	Pro	Lys
80					85					90					95

Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser
 100 105 110
 Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu
 115 120 125
 Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr
 130 135 140
 Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp
 145 150 155
 Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
 160 165 170 175
 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys
 180 185 190
 Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu
 195 200 205
 Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro
 210 215 220
 Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys
 225 230 235
 Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys
 240 245 250 255
 Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly
 260 265 270
 Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr
 275 280 285
 Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val
 290 295 300
 Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser
 305 310 315

EXHIBIT B

CCCCCCCCGC CTCTCCAAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCCTCGCC	60
CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCGGGTIT CCTGTGAGGC	120
TTTTACCTGA CACCCGCCGC CTTTCCCCGG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG	180
GGAACGCGGA GCCCCGGACC CGCTCCCGCC GCCTCCGGCT CGCCCAGGGG GGGTCGCCGG	240
GAGGAGCCCG GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGGC GCCCCGCGCC	300
CCACCCCTGC CCCCCGCCAGC GGACCGGTCC CCCACCCCGG GTCCTTCCAC C ATG CAC Met His 1	357
TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Ala Leu 5 10 15	405
CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC GCC TTC GAG TCC Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser 20 25 30	453
GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala 35 40 45 50	501
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val 55 60 65	549
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG Asp Glu Leu Met Thr Val Leu Tyr Trp Glu Tyr Trp Lys Met Tyr Lys 70 75 80	597
TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn 85 90 95	645
CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr 100 105 110	693
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln 115 120 125 130	741
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val 135 140 145	789
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys 150 155 160	837
GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr 165 170 175	885
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln 180 185 190	933
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg 195 200 205 210	981

TGC	ATG	TCT	AAA	CTG	GAT	GTT	TAC	AGA	CAA	GTT	CAT	TCC	ATT	ATT	AGA	1029
Cys	Met	Ser	Lys	Leu	Asp	Val	Tyr	Arg	Gln	Val	His	Ser	Ile	Ile	Arg	
				215					220					225		
CGT	TCC	CTG	CCA	GCA	ACA	CTA	CCA	CAG	TGT	CAG	GCA	GCG	AAC	AAG	ACC	1077
Arg	Ser	Leu	Pro	Ala	Thr	Leu	Pro	Gln	Cys	Gln	Ala	Ala	Asn	Lys	Thr	
			230					235					240			
TGC	CCC	ACC	AAT	TAC	ATG	TGG	AAT	AAT	CAC	ATC	TGC	AGA	TGC	CTG	GCT	1125
Cys	Pro	Thr	Asn	Tyr	Met	Trp	Asn	Asn	His	Ile	Cys	Arg	Cys	Leu	Ala	
		245					250					255				
CAG	GAA	GAT	TTT	ATG	TTT	TCC	TCG	GAT	GCT	GGA	GAT	GAC	TCA	ACA	GAT	1173
Gln	Glu	Asp	Phe	Met	Phe	Ser	Ser	Asp	Ala	Gly	Asp	Asp	Ser	Thr	Asp	
	260					265					270					
GGA	TTC	CAT	GAC	ATC	TGT	GGA	CCA	AAC	AAG	GAG	CTG	GAT	GAA	GAG	ACC	1221
Gly	Phe	His	Asp	Ile	Cys	Gly	Pro	Asn	Lys	Glu	Leu	Asp	Glu	Glu	Thr	
	275			280						285					290	
TGT	CAG	TGT	GTC	TGC	AGA	GCG	GGG	CTT	CGG	CCT	GCC	AGC	TGT	GGA	CCC	1269
Cys	Gln	Cys	Val	Cys	Arg	Ala	Gly	Leu	Arg	Pro	Ala	Ser	Cys	Gly	Pro	
				295					300					305		
CAC	AAA	GAA	CTA	GAC	AGA	AAC	TCA	TGC	CAG	TGT	GTC	TGT	AAA	AAC	AAA	1317
His	Lys	Glu	Leu	Asp	Arg	Asn	Ser	Cys	Gln	Cys	Val	Cys	Lys	Asn	Lys	
			310					315					320			
CTC	TTC	CCC	AGC	CAA	TGT	GGG	GCC	AAC	CGA	GAA	TTT	GAT	GAA	AAC	ACA	1365
Leu	Phe	Pro	Ser	Gln	Cys	Gly	Ala	Asn	Arg	Glu	Phe	Asp	Glu	Asn	Thr	
		325				330						335				
TGC	CAG	TGT	GTA	TGT	AAA	AGA	ACC	TGC	CCC	AGA	AAT	CAA	CCC	CTA	AAT	1413
Cys	Gln	Cys	Val	Cys	Lys	Arg	Thr	Cys	Pro	Arg	Asn	Gln	Pro	Leu	Asn	
		340				345					350					
CCT	GGA	AAA	TGT	GCC	TGT	GAA	TGT	ACA	GAA	AGT	CCA	CAG	AAA	TGC	TTG	1461
Pro	Gly	Lys	Cys	Ala	Cys	Glu	Cys	Thr	Glu	Ser	Pro	Gln	Lys	Cys	Leu	
				360					365					370		
TTA	AAA	GGA	AAG	AAG	TTC	CAC	CAC	CAA	ACA	TGC	AGC	TGT	TAC	AGA	CGG	1509
Leu	Lys	Gly	Lys	Lys	Phe	His	His	Gln	Thr	Cys	Ser	Cys	Tyr	Arg	Arg	
				375				380						385		
CCA	TGT	ACG	AAC	CGC	CAG	AAG	GCT	TGT	GAG	CCA	GGA	TTT	TCA	TAT	AGT	1557
Pro	Cys	Thr	Asn	Arg	Gln	Lys	Ala	Cys	Glu	Pro	Gly	Phe	Ser	Tyr	Ser	
			390				395					400				
GAA	GAA	GTG	TGT	CGT	TGT	GTC	CCT	TCA	TAT	TGG	AAA	AGA	CCA	CAA	ATG	1605
Glu	Glu	Val	Cys	Arg	Cys	Val	Pro	Ser	Tyr	Trp	Lys	Arg	Pro	Gln	Met	
		405				410						415				
AGC	TAAGATTGTA	CTGTTTTCCA	GTTTCATCGAT	TTTCTATTAT	GGAAAACTGT											1658
Ser																
GTTGCCACAG	TAGAACTGTC	TGTGAACAGA	GAGACCCTTG	TGGGTCCATG	CTAACAAAGA											1718
CAAAAGTCTG	TCTTTCCTGA	ACCATGTGGA	TAACTTTACA	GAAATGGACT	GGAGCTCATC											1778
TGCAAAAGGC	CTCTTGTA	AAAGACTGTTTT	CTGCCAATGA	CCAAACAGCC	AAGATTTTCC											1838
TCTTGTGATT	TCTTTAAAG	AATGACTATA	TAATTTATTT	CCACTAAAAA	TATTGTTTCT											1898
GCAITTCATTT	TTATAGCAAC	AACAATTGGT	AAAACCTCACT	GTGATCAATA	TTTTTATATC											1958
ATGCAAAATA	TGTTTAAAT	AAAATGAAAA	TTGTATTAT													1997

EXHIBIT C

Mouse VEGF-C cDNA and deduced amino acid sequence

GCGGCCGCGT	CGACGCAAAA	GTTGCGAGCC	GCCGAGTCCC	GGGAGACGCT	CGCCCAGGGG	60
GGTCCCCGGG	AGGAAACCAC	GGGACAGGGA	CCAGGAGAGG	ACCTCAGCCT	CACGCCCCAG	120
CCTGCGCCAG	CCAACGGACC	GGCCTCCCTG	CTCCCGGTCC	ATCCACC	ATG CAC TTG Met His Leu 1	176
CTG TGC TTC	TTG TCT CTG	GCG TGT TCC	CTG CTC GGC	GCT GCG CTG	ATC	224
Leu Cys Phe	Leu Ser Leu	Ala Cys Ser	Leu Leu Ala	Ala Ala Leu	Ile	
5	10	15	20	25	30	
CCC AGT CCG	CGC GAG GCG	CCC GCC ACC	GTC GCC GGC	TTC GAG TCG	GGA	272
Pro Ser Pro	Arg Glu Ala	Pro Ala Thr	Val Ala Ala	Phe Glu Ser	Gly	
20	25	30	35	40	45	
CTG GGC TTC	TCG GAA GCG	GAG CCC GAC	GGG GGC GAG	GTC AAG GCT	TTT	320
Leu Gly Phe	Ser Glu Ala	Glu Pro Asp	Gly Gly Glu	Val Lys Ala	Phe	
40	45	50	55	60	65	
GAA GGC AAA	GAC CTG GAG	GAG CAG TTG	CGG TCT GTG	TCC AGC GTA	GAT	368
Glu Gly Lys	Asp Leu Glu	Glu Gln Leu	Arg Ser Val	Ser Ser Val	Asp	
55	60	65	70	75	80	
GAG CTG ATG	TCT GTC CTG	TAC CCA GAC	TAC TGG AAA	ATG TAC AAG	TGC	416
Glu Leu Met	Ser Val Leu	Tyr Pro Asp	Tyr Trp Lys	Met Tyr Lys	Cys	
70	75	80	85	90	95	
CAG CTG CGG	AAA GGC GGC	TGG CAG CAG	CCC ACC CTC	AAT ACC AGG	ACA	464
Gln Leu Arg	Lys Gly Gly	Trp Gln Gln	Pro Thr Leu	Asn Thr Arg	Thr	
85	90	95	100	105	110	
GGG GAC AGT	GTA AAA TTT	GCT GCT GCA	CAT TAT AAC	ACA GAG ATC	CTG	512
Gly Asp Ser	Val Lys Phe	Ala Ala Ala	His Tyr Asn	Thr Glu Ile	Leu	
100	105	110	115	120	125	
AAA AGT ATT	GAT AAT GAG	TGG AGA AAG	ACT CAA TGC	ATG CCA CGT	GAG	560
Lys Ser Ile	Asp Asn Glu	Trp Arg Lys	Thr Gln Cys	Met Pro Arg	Glu	
120	125	130	135	140	145	
GTG TGT ATA	GAT GTG GGG	AAG GAG TTT	GGA GCA GCC	ACA AAC ACC	TTC	608
Val Cys Ile	Asp Val Gly	Lys Glu Phe	Gly Ala Ala	Thr Asn Thr	Phe	
135	140	145	150	155	160	
TTT AAA CCT	CCA TGT GTG	TCC GTC TAC	AGA TGT GGG	GGT TGC TGC	AAC	656
Phe Lys Pro	Pro Cys Val	Ser Val Tyr	Arg Cys Gly	Gly Cys Cys	Asn	
150	155	160	165	170	175	
AGC GAG GGG	CTG CAG TGC	ATG AAC ACC	AGC ACA GGT	TAC CTC AGC	AAG	704
Ser Glu Gly	Leu Gln Cys	Met Asn Thr	Ser Thr Gly	Tyr Leu Ser	Lys	
165	170	175	180	185	190	
ACG TTG TTT	GAA ATT ACA	GTG CCT CTC	TCA CAA GGC	CCC AAA CCA	GTC	752
Thr Leu Phe	Glu Ile Thr	Val Pro Leu	Ser Gln Gly	Pro Lys Pro	Val	
180	185	190	195	200	205	
ACA ATC AGT	TTT GCC AAT	CAC ACT TCC	TGC CGG TGC	ATG TCT AAA	CTG	800
Thr Ile Ser	Phe Ala Asn	His Thr Ser	Cys Arg Cys	Met Ser Lys	Leu	
200	205	210	215	220	225	
GAT GTT TAC	AGA CAA GTT	CAT TCA ATT	ATT AGA CGT	TCT CTG CCA	GCA	848
Asp Val Tyr	Arg Gln Val	His Ser Ile	Arg Arg Ser	Leu Pro Ala		
215	220	225	230	235	240	


ACA TTA CCA CAG TGT CAG GCA GCT AAC AAG ACA TGT CCA ACA AAC TAT Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr 230 235 240	896
GTG TGG AAT AAC TAC ATG TGC CGA TGC CTG GCT CAG CAG GAT TTT ATC Val Trp Asn Asn Tyr Met Cys Arg Cys Leu Ala Gln Gln Asp Phe Ile 245 250 255	944
TTT TAT TCA AAT GTT GAA GAT GAC TCA ACC AAT GGA TTC CAT GAT GTC Phe Tyr Ser Asn Val Glu Asp Asp Ser Thr Asn Gly Phe His Asp Val 260 265 270 275	992
TGT GGA CCC AAC AAG GAG CTG GAT GAA GAC ACC TGT CAG TGT GTC TGC Cys Gly Pro Asn Lys Glu Leu Asp Glu Asp Thr Cys Gln Cys Val Cys 280 285 290	1040
AAG GGG GGG CTT CGG CCA TCT AGT TGT GGA CCC CAC AAA GAA CTA GAT Lys Gly Gly Leu Arg Pro Ser Ser Cys Gly Pro His Lys Glu Leu Asp 295 300 305	1088
AGA GAC TCA TGT CAG TGT GTC TGT AAA AAC AAA CTT TTC CCT AAT TCA Arg Asp Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Pro Asn Ser 310 315 320	1136
TGT GGA GCC AAC AGG GAA TTT GAT GAG AAT ACA TGT CAG TGT GTA TGT Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys 325 330 335	1184
AAA AGA ACG TGT CCA AGA AAT CAG CCC CTG AAT CCT GGG AAA TGT GCC Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala 340 345 350 355	1232
TGT GAA TGT ACA GAA AAC ACA CAG AAG TGC TTC CTT AAA GGG AAG AAG Cys Glu Cys Thr Glu Asn Thr Gln Lys Cys Phe Leu Lys Gly Lys Lys 360 365 370	1280
TTC CAC CAT CAA ACA TGC AGT TGT TAC AGA AGA CCG TGT GCG AAT CGA Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Ala Asn Arg 375 380 385	1328
CTG AAG CAT TGT GAT CCA GGA CTG TCC TTT AGT GAA GAA GTA TGC CGC Leu Lys His Cys Asp Pro Gly Leu Ser Phe Ser Glu Glu Val Cys Arg 390 395 400	1376
TGT GTC CCA TCG TAT TGG AAA AGG CCA CAT CTG AAC TAAGATCATA Cys Val Pro Ser Tyr Trp Lys Arg Pro His Leu Asn 405 410 415	1422
CCAGTTTTC A GTCAGTCACA GTCATTTACT CTCTTGAAGA CTGTTGGAAC AGCACTTAGC	1482
ACTGTCTATG CACAGAAAGA CTCGTGGGA CCACATGGTA ACAGAGGCCC AAGTCTGTGT	1542
TTATTGAACC ATGTGGATTA CTGCGGGAGA GGA CTGGCAC TCATGTGCAA AAAAAACCTC	1602
TTCAAGACT GGT TTTCTGC CAGGGACCAG ACAGCTGAGG TTTTCTCTT GTGATTTAAA	1662
AAAAGAAATGA CTATATAAT TATTTCCACT AAAAATATTG TTCCTGCATT CATTTTATA	1722
GCAATAACAA TTGGTAAAGC TCACTGTGAT CAGTATTTTT ATAACATGCA AACTATGTT	1782
TAAATAAAAA TGAAATTTGT ATTATAAAAA AAAAAAAAAA AAAAAAAAAA GCTT	1836

EXHIBIT D

Quail VEGF-C

GCCCCCGCCG AGCGCTCCGC GCGCAGCCGC CGGGCCGGGC CGGCCCCGCG AGGGCGCGCT	60
GCGAGCGGCC ACTGGGTCTT GCTTCCCTCC TTCCTCTCCC TCCTCCTCCT CCTCCTTCTC	120
TCTGCGCTTT CCACCGCTCC CGAGCGAGCG CACGCTCGGA TGTCCGGTTT CCTGGTGGGT	180
TTTTTACCTG GCAAAGTCCG GATAACTTCG GTGAGAATTT GCAAAGAGGC TGGGAGCTCC	240
CCTGCAGGCG TCTGGGAGCT GCTGCCGCGC TCGCATCTTC TCCATCCCGC GGATTTTACT	300
GCCTTGGATA TTGCGAGGGG AGGGAGGGGG GTGAGGACAG CAAAAGAAA GGGGTGGGGG	360
GGGGGAGAGA AAAGGAAAAG AAGGAGCCTC GGAATTGTGC CCGCATTCTT GCGCTGCCCC	420
GCGGCCCCCC TCCGCTCTGC CATCTCCGCA CA ATG CAC TTG CTG GAG ATG CTC	473
Met His Leu Leu Glu Met Leu	
1 5	
TCC CTG GGC TGC TGC CTC GCT GCT GGC GCC GTG CTC CTG GGA CCC CGG	521
Ser Leu Gly Cys Cys Leu Ala Ala Gly Ala Val Leu Leu Gly Pro Arg	
10 15 20	
CAG CCG CCC GTC GCC GCC GCC TAC GAG TCC GGG CAC GGC TAC TAC GAG	569
Gln Pro Pro Val Ala Ala Ala Tyr Glu Ser Gly His Gly Tyr Tyr Glu	
25 30 35	
GAG GAG CCC GGT GCC GGG GAA CCC AAG GCT CAT GCA AGC AAA GAC CTG	617
Glu Glu Pro Gly Ala Gly Glu Pro Lys Ala His Ala Ser Lys Asp Leu	
40 45 50 55	
GAA GAG CAG TTG CGA TCT GTG TCC AGT GTG GAT GAA CTC ATG ACA GTA	665
Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp Glu Leu Met Thr Val	
60 65 70	
CTT TAC CCA GAA TAC TGG AAA ATG TTC AAA TGT CAG TTG AGG AAA GGA	713
Leu Tyr Pro Glu Tyr Trp Lys Met Phe Lys Cys Gln Leu Arg Lys Gly	
75 80 85	
GGT TGG CAA CAC AAC AGG GAA CAC TCC AGC TCT GAT ACA AGA TCA GAT	761
Gly Trp Gln His Asn Arg Glu His Ser Ser Ser Asp Thr Arg Ser Asp	
90 95 100	
GAT TCA TTG AAA TTT GCC GCA GCA CAT TAT AAT GCA GAG ATC CTG AAA	809
Asp Ser Leu Lys Phe Ala Ala His Tyr Asn Ala Glu Ile Leu Lys	
105 110 115	
AGT ATT GAT ACT GAA TGG AGA AAA ACC CAG GGC ATG CCA CGT GAA GTG	857
Ser Ile Asp Thr Glu Trp Arg Lys Thr Gln Gly Met Pro Arg Glu Val	
120 125 130 135	
TGT GTG GAT TTG GGG AAA GAG TTT GGA GCA ACT ACA AAC ACC TTC TTT	905
Cys Val Asp Leu Gly Lys Glu Phe Gly Ala Thr Thr Asn Thr Phe Phe	
140 145 150	
AAA CCC CCG TGT GTA TCC ATC TAC AGA TGT GGA GGT TGC TGC AAT AGT	953
Lys Pro Pro Cys Val Ser Ile Tyr Arg Cys Gly Gly Cys Cys Asn Ser	
155 160 165	
GAA GGA CTC CAG TGT ATG AAT ATC AGC ACA AAT TAC ATC AGC AAG ACA	1001
Glu Gly Leu Gln Cys Met Asn Ile Ser Thr Asn Tyr Ile Ser Lys Thr	
170 175 180	

TTG TTT GAG ATT ACA GTG CCT CTC TCT CAT GGC CCC AAA CCT GTA ACA Leu Phe Glu Ile Thr Val Pro Leu Ser His Gly Pro Lys Pro Val Thr 185 190 195	1049
GTC AGT TTT GCC AAT CAC ACG TCC TGC CGA TGC ATG TCT AAG TTG GAT Val Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp 200 205 210 215	1097
GTT TAC AGA CAA GTT CAT TCT ATC ATA AGA CGT TCC TTG CCA GCA ACA Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr 220 225 230	1145
CAA ACT CAG TGT CAT GTG GCA AAC AAG ACC TGT CCA AAA AAT CAT GTC Gln Thr Gln Cys His Val Ala Asn Lys Thr Cys Pro Lys Asn His Val 235 240 245	1193
TGG AAT AAT CAG ATT TGC AGA TGC TTA GCA CAG CAC GAT TTT GGT TTC Trp Asn Asn Gln Ile Cys Arg Cys Leu Ala Gln His Asp Phe Gly Phe 250 255 260	1241
TCT TCT CAC CTT GGA GAT TCT GAC ACA TCT GAA GGA TTC CAT ATT TGT Ser Ser His Leu Gly Asp Ser Asp Thr Ser Glu Gly Phe His Ile Cys 265 270 275	1289
GGG CCC AAC AAA GAG CTG GAT GAA GAA ACC TGT CAA TGC GTC TGC AAA Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Lys 280 285 290 295	1337
GGA GGT GTG CGG CCC ATA AGC TGT GGC CCT CAC AAA GAA CTA GAC AGG Gly Gly Val Arg Pro Ile Ser Cys Gly Pro His Lys Glu Leu Asp Arg 300 305 310	1385
GCA TCA TGT CAG TGC ATG TGC AAA AAC AAA CTG CTC CCC AGT TCC TGT Ala Ser Cys Gln Cys Met Cys Lys Asn Lys Leu Leu Pro Ser Ser Cys 315 320 325	1433
GGG CCT AAC AAA GAA TTT GAT GAA GAA AAG TGC CAG TGT GTA TGT AAA Gly Pro Asn Lys Glu Phe Asp Glu Glu Lys Cys Gln Cys Val Cys Lys 330 335 340	1481
AAG ACC TGT CCC AAA CAT CAT CCA CTA AAT CCT GCA AAA TGC ATC TGC Lys Thr Cys Pro Lys His His Pro Leu Asn Pro Ala Lys Cys Ile Cys 345 350 355	1529
GAA TGT ACA GAA TCT CCC AAT AAA TGT TTC TTA AAA GGA AAA AGA TTT Glu Cys Thr Glu Ser Pro Asn Lys Cys Phe Leu Lys Gly Lys Arg Phe 360 365 370 375	1577
CAT CAC CAG ACA TGC AGT TGT TAC AGA CCA CCA TGT ACA GTC CGA ACG His His Gln Thr Cys Ser Cys Tyr Arg Pro Pro Cys Thr Val Arg Thr 380 385 390	1625
AAA CGC TGT GAT GCT GGA TTT CTG TTA GCT GAA GAA GTG TGC CGC TGT Lys Arg Cys Asp Ala Gly Phe Leu Leu Ala Glu Glu Val Cys Arg Cys 395 400 405	1673
GTA CGC ACA TCT TGG AAA AGA CCA CTT ATG AAT TAAGCGAAGA AAGCACTACT Val Arg Thr Ser Trp Lys Arg Pro Leu Met Asn 410 415	1726
CGCTATATAG TGTCG	1741

Notice of Allowability	Application No. 08/510,133	Inventor(s) ALITALO et al.	
	Examiner Christine Saoud	Group Art Unit 1647	

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance and Issue Fee Due or other appropriate communication will be mailed in due course.

☒ This communication is responsive to amendment of 26 July 2000.

☒ The allowed claim(s) is/are 29-57, renumbered as 1-29.

☐ The drawings filed on _____ are acceptable.

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been
☐ received.
☐ received in Application No. (Series Code/Serial Number) _____
☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE "DATE MAILED" of this Office action. Failure to timely comply will result in ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.

☒ Applicant MUST submit NEW FORMAL DRAWINGS

☐ because the originally filed drawings were declared by applicant to be informal.
☒ including changes required by the Notice of Draftsperson's Patent Drawing Review, PTO-948, attached hereto or to Paper No. 7.
☐ including changes required by the proposed drawing correction filed on _____, which has been approved by the examiner.
☐ including changes required by the attached Examiner's Amendment/Comment.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the reverse side of the drawings. The drawings should be filed as a separate paper with a transmittal letter addressed to the Official Draftsperson.

☐ Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Any response to this letter should include, in the upper right hand corner, the APPLICATION NUMBER (SERIES CODE/SERIAL NUMBER). If applicant has received a Notice of Allowance and Issue Fee Due, the ISSUE BATCH NUMBER and DATE of the NOTICE OF ALLOWANCE should also be included.

Attachment(s)

☐ Notice of References Cited, PTO-892
☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 18
☒ Notice of Draftsperson's Patent Drawing Review, PTO-948
☐ Notice of Informal Patent Application, PTO-152
☐ Interview Summary, PTO-413
☐ Examiner's Amendment/Comment
☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
☐ Examiner's Statement of Reasons for Allowance

CHRISTINE SAOUD
PATENT EXAMINER
Christine Saoud

EXHIBIT E

VEGF-C alignment

	1				50
Hum	HMLLGFFSVA	CSLLAAALLP	GPRAAPAAAA	AFESGLDLS	AEPDAGEATA
Mou	MHLLCFLSLA	CSLLAAALIP	SPREAPATVA	AFESGLGFSE	AEPDGGEVKA
Qua	MHLEMLSLG	CCLAAGAVLL	GPRQPPVA.A	AYESGHGYE	EPPGAGEPKA
	51				100
Hum	YASKDLEEQL	RSVSSVDELM	TVLYPEYWKM	YKCQLRKGGW	QHNREQANLN
Mou	FEGKDLEEQL	RSVSSVDELM	SVLYPDYWKH	YKCQLRKGGW	Q....OPTLN
Qua	HASKDLEEQL	RSVSSVDELM	TVLYPEYWKM	FKCQLRKGGW	QHNREHSSSD
	101				150
Hum	SRTEETIKFA	AAHYNTEILK	SIDNEWRTQ	CMPEVVCIDV	GKEFGVATNT
Mou	TRTGDSVKFA	AAHYNTEILK	SIDNEWRTQ	CMPEVVCIDV	GKEFGAATNT
Qua	TRSDDSLKFA	AAHYNAEILK	SIDTEWRKTQ	GMPEVVCIDL	GKEFGATTNT
	151				200
Hum	FFKPPCVSVY	RCGGCCNSEG	LQCMNTTSTY	LSKTLFEITV	PLSQGPKPVT
Mou	FFKPPCVSVY	RCGGCCNSEG	LQCMNTSTGY	LSKTLFEITV	PLSQGPKPVT
Qua	FFKPPCVSIY	RCGGCCNSEG	LQCMNISTNY	ISKTLFEITV	PLSHGPKPVT
	201				250
Hum	ISFANHTSCR	CMSKLDVYRQ	VHSIIRSLP	ATLPQCCAAN	KTCPTNYMWN
Mou	ISFANHTSCR	CMSKLDVYRQ	VHSIIRSLP	ATLPQCCAAN	KTCPTNYVWN
Qua	VSFANHTSCR	CMSKLDVYRQ	VHSIIRSLP	ATQTQCHVAN	KTCPKNHVWN
	251				300
Hum	NHICRCLAQE	DFMFSSDAGD	DSTDGFHDIC	GPKNELDEET	CQCVCRAGLR
Mou	NYMCRCCLAQQ	DFIFYSNVED	DSTNGPHDVC	GPKNELDEET	CQCVCCKGGLR
Qua	NQICRCLAQH	DFGFSSHLGD	SOTSEGFHIC	GPKNELDEET	CQCVCCKGGVR
	301				350
Hum	PASCGPHKEL	DRNSCQCVCX	NKLFPSQCGA	NREFDENTCQ	CVCKRTCPRN
Mou	PSSCGPHKEL	DRDSCQCVCX	NKLFPSQCGA	NREFDENTCQ	CVCKRTCPRN
Qua	PISCGPHKEL	DRASCQCMCK	NKLLPSSCGP	NKEFDEEKCQ	CVCKKTCPKH
	351				400
Hum	QPLNPGKCAC	ECTESPQKCL	LKGKKFHHQT	CSCYRRPCTN	RQKACEPGFS
Mou	QPLNPGKCAC	ECTENTQKCF	LKGKKFHHQT	CSCYRRPCAN	RLKHCDPGLS
Qua	HPLNPAKCIC	ECTESPNKCF	LKGKRFHHQT	CSCYRPPCTV	RTKRCDAGFL
	401		420		
Hum	YSEEVCRCPV	SYWKRQMS*			
Mou	FSEEVCRCPV	SYWKRPHLN.			
Qua	LAEEVCRCPV	TSWKRPLMN*			



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

NOTICE OF ALLOWANCE AND ISSUE FEE DUE

APPLICATION NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
First Named Applicant				
TITLE OF INVENTION				

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED.

THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED.

HOW TO RESPOND TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

- A. If the status is changed, pay twice the amount of the FEE DUE shown above and notify the Patent and Trademark Office of the change in status, or
- B. If the status is the same, pay the FEE DUE shown above.

If the SMALL ENTITY is shown as NO:

- A. Pay FEE DUE shown above, or
- B. File verified statement of Small Entity Status before, or with, payment of 1/2 the FEE DUE shown above.

II. Part B-Issue Fee Transmittal should be completed and returned to the Patent and Trademark Office (PTO) with your ISSUE FEE. Even if the ISSUE FEE has already been paid by charge to deposit account, Part B Issue Fee Transmittal should be completed and returned. If you are charging the ISSUE FEE to your deposit account, section "4b" of Part B-Issue Fee Transmittal should be completed and an extra copy of the form should be submitted.

III. All communications regarding this application must give application number and batch number. Please direct all communications prior to issuance to Box ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PATENT AND TRADEMARK OFFICE COPY

NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

PTO Draftpersons review all originally filed drawings regardless of whether they are designated as formal or informal. Additionally, patent Examiners will review the drawings for compliance with the regulations. Direct telephone inquiries concerning this review to the Drawing Review Branch, 703-305-8404.

The drawings filed (insert date) 08/01/95, are
A. ☒ not objected to by the Draftsperson under 37 CFR 1.84 or 1.152.
B. ☒ objected to by the Draftsperson under 37 CFR 1.84 or 1.152 as indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawings must be submitted according to the instructions on the back of this Notice.

1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings:
Black ink. Color.

- ☐ Not black solid lines. Fig(s) _____
- ☐ Color drawings are not acceptable until petition is granted. Fig(s) _____

2. PHOTOGRAPHS. 37 CFR 1.84(b) (3 SETS REQUIRED)

- ☒ Photographs are not acceptable until petition is granted. Fig(s) 4-8, 11-12
- ☒ Photographs not properly mounted (must use bristol board or photographic double-weight paper). Fig(s) 4-8, 11-12
- ☒ Poor quality (half-tone). Fig(s) 4-8, 11-12

3. GRAPHIC FORMS. 37 CFR 1.84(d)

- ☐ Chemical or mathematical formula not labeled as separate figure. Fig(s) _____
- ☐ Group of waveforms not presented as a single figure, using common vertical axis with time extending along horizontal axis. Fig(s) _____
- ☐ Individuals waveform not identified with a separate letter designation adjacent to the vertical axis. Fig(s) _____

4. TYPE OF PAPER. 37 CFR 1.84(c)

- ☐ Paper not flexible, strong, white, smooth, nonshiny, and durable. Sheet(s) _____
- ☐ Erasures, alterations, overwritings, interlineations, cracks, creases, and folds copy machine marks not accepted. Fig(s) _____
- ☐ Mylar, velum paper is not acceptable (too thin). Fig(s) _____

5. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable sizes:

- ☐ 21.6 cm. by 35.6 cm. (8 1/2 by 14 inches)
- ☐ 21.6 cm. by 33.1 cm. (8 1/2 by 13 inches)
- ☐ 21.6 cm. by 27.9 cm. (8 1/2 by 11 inches)
- ☐ 21.0 cm. by 29.7 cm. (DIN size A4)
- ☐ All drawing sheets not the same size. Sheet(s) _____
- ☐ Drawing sheet not an acceptable size. Sheet(s) _____

6. MARGINS. 37 CFR 1.84(g): Acceptable margins:

Paper size

21.6 cm. X 35.6 cm. (8 1/2 X 14 inches)	21.6 cm. X 33.1 cm. (8 1/2 X 13 inches)	21.6 cm. X 27.9 cm. (8 1/2 X 11 inches)	21.0 cm. X 29.7 cm. (DIN Size A4)
T 5.1 cm. (2")	2.5 cm. (1")	2.5 cm. (1")	2.5 cm.
L .64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")	2.5 cm.
R .64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")	1.5 cm.
B .64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")	1.0 cm.

Margins do not conform to chart above.

Sheet(s) Fig. 2, 9, 10☒ Top (T) ☒ Left (L) ☐ Right (R) ☐ Bottom (B)

7. VIEWS. 37 CFR 1.84(h)

REMINDER: Specification may require revision to correspond to drawing changes.

- ☐ All views not grouped together. Fig(s) _____
- ☐ Views connected by projection lines or lead lines. Fig(s) _____
- ☐ Partial views. 37 CFR 1.84(h) 2

☐ View and enlarged view not labeled separately or properly. Fig(s) _____☐ Sectional views. 37 CFR 1.84 (h) 3☐ Hatching not indicated for sectional portions of an object. Fig(s) _____☐ Cross section not drawn same as view with parts in cross section with regularly spaced parallel oblique strokes. Fig(s) _____

8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(i)

- ☐ Words do not appear on a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) _____

9. SCALE. 37 CFR 1.84(k)

- ☐ Scale not large enough to show mechanism with crowding when drawing is reduced in size to two-thirds in reproduction. Fig(s) _____
- ☐ Indication such as "actual size" or scale 1/2" not permitted. Fig(s) _____

10. CHARACTER OF LINES, NUMBERS, & LETTERS. 37 CFR 1.84(l)

- ☒ Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (except for color drawings). Fig(s) A, L

11. SHADING. 37 CFR 1.84(m)

- ☐ Solid black shading areas not permitted. Fig(s) _____
- ☐ Shade lines, pale, rough and blurred. Fig(s) _____

12. NUMBERS, LETTERS, & REFERENCE CHARACTERS. 37 CFR 1.84(p)

- ☐ Numbers and reference characters not plain and legible. 37 CFR 1.84(p)(1) Fig(s) _____
- ☐ Numbers and reference characters not oriented in same direction as the view. 37 CFR 1.84(p)(1) Fig(s) _____
- ☐ English alphabet not used. 37 CFR 1.84(p)(2) Fig(s) _____
- ☒ Numbers, letters, and reference characters do not measure at least .32 cm. (1/8 inch) in height. 37 CFR(p)(3) Fig(s) 2, 9, 10

13. LEAD LINES. 37 CFR 1.84(q)

- ☐ Lead lines cross each other. Fig(s) _____
- ☐ Lead lines missing. Fig(s) _____

14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(i)

- ☐ Sheets not numbered consecutively, and in Arabic numerals, beginning with number 1. Sheet(s) _____

15. NUMBER OF VIEWS. 37 CFR 1.84(u)

- ☐ Views not numbered consecutively, and in Arabic numerals, beginning with number 1. Fig(s) _____
- ☐ View numbers not preceded by the abbreviation Fig. Fig(s) _____

16. CORRECTIONS. 37 CFR 1.84(w)

- ☐ Corrections not made from prior PTO-948. Fig(s) _____

17. DESIGN DRAWING. 37 CFR 1.152

- ☐ Surface shading shown not appropriate. Fig(s) _____
- ☐ Solid black shading not used for color contrast. Fig(s) _____

COMMENTS:

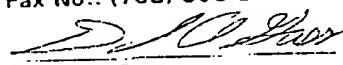
Nov. 2. 2000 3:42PM MARSHALL, O'TOOLE

No. 5292 P. 2/2
From: 0819

PATENT
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.
Serial No: 08/510,133
Filed: August 1, 1995
Title: Receptor Ligand
Group Art Unit: 1646
Examiner: Christine Saoud

) I hereby certify that this paper is
) being sent via facsimile to:
) Commissioner for Patents,
) Washington, D.C., 20231 on this
) date: Date: November 2, 2000.
) Fax No.: (703) 305-3014
) 
) David A. Gass
) Registration No. 38,153
) Attorney for Applicants

Commissioner for Patents
Washington, D.C. 20231

CHANGE OF ADDRESS

Sir:

The undersigned is an attorney of record in this case. Please mail all correspondence in this case to the undersigned at the address below :

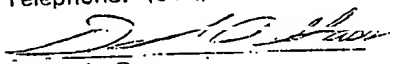
David A. Gass
Marshall, O'Toole, Gerstein, Murray & Borun
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402

The attorney's phone number is (312) 474-6300.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

Dated: November 2, 2000


David A. Gass
Registration No. 38,153

OK to Enter

B.3

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled RECEPTOR LIGAND, by inventor(s) Kari Alitalo and Vladimir Joukov

described in

- ☐ The specification filed herewith.
- ☒ Application Serial No. 08/510,133, filed August 1, 1995.
- ☐ Patent No. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization regarding the above-identified invention. If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights in the invention is listed below and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

OK to Enter

FULL NAME: Helsinki University Licensing, Ltd.
ADDRESS: Viikinkaari 8 A, FIN-00710 Helsinki, Finland
☐ INDIVIDUAL ☒ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME: _____
ADDRESS: _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that

such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

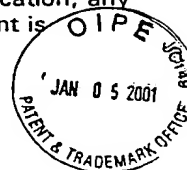
NAME OF PERSON SIGNING: Edward A McDermott

TITLE IN ORGANIZATION: President

ADDRESS OF PERSON SIGNING: 1345 Avenue of the Americas, New York
NY 10105

SIGNATURE: *Edward A. McDermott*

Date: March 2, 1997





PATENT

Attorney's Docket No: 28967/32863

Applicant or Patentee: Kari Alitalo and Vladimir Joukov
Serial or Patent No: 08/510,133
Filed or Issued: August 1, 1995
For: Receptor Ligand

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(c)) -- SMALL BUSINESS CONCERN

I hereby declare that I am

- ☐ The owner of the small business concern identified below:
- ☒ An official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: Helsinki University Licensing, Ltd.

ADDRESS OF BUSINESS: Viikinkaari 8 A, FIN-00710 Helsinki, Finland

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to, and remain with, the small business concern identified above with regard to the invention, entitled Receptor Ligand, by inventor(s) Kari Alitalo and Vladimir Joukov,

described in

- ☐ The specification filed herewith.
- ☒ Application Serial No. 08/510,133, filed August 1, 1995.
- ☐ Patent No. _____, issued _____.



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	08/01 FILING DATE	ALIT: 0	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO. 328
--------------------	-------------------	---------	-----------------------	-------------------------

7542/0130

FRANK S. DIGIGLIO
SCULLY SCOTT MURPHY & PRESSER
400 GARDEN CITY PLAZA
GARDEN CITY NY 11530

SAC/EXAMINER	
#35	
ART UNIT 7	PAPER NUMBER

01/30/01

DATE MAILED:

Response to Rule 312 Communication

- ☐ The petition filed on _____ under 37 CFR 1.312(b) is granted. The paper has been forwarded to the examiner for consideration on the merits.

Director,
Patent Examining Group _____

- ☒ The amendment filed on 1/3/01 under 37 CFR 1.312 has been considered, and has been:
- ☒ entered.
 - ☐ entered as directed to matters of form not affecting the scope of the invention (Order 3311).
 - ☐ disapproved. See explanation below.
 - ☐ entered in part. See explanation below.

Kenna Scott
Publishing Division

The remaining amendments to the specification merely conform the specification to the formal drawings submitted concurrently herewith. Figures 2, 6, 9 and 10 were prepared on multiple sheets and/or renumbered in order to comply with the Draftsman's requirements. The specification has been amended to reflect the fact that these figures will be multiple pages in the issued patent.

These amendment add no new matter and do not raise any new patentability issues that would require any substantive examination by the Examiner.

In view of the foregoing, the applicant respectfully requests the granting of the amendment after allowance.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By: 

David A. Gass
Registration No. 38,153
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

January 3, 2001

the allowed claims. The Patent Office is authorized to charge any fee associated with this amendment to Deposit Account No. 13-2855.

The amendment to page 1 amounts to a cancellation of a priority claim to an application that was filed in November, 1994. The sole purpose behind cancellation of the priority claim is to maximize patent term of the eventual patent, because it is the Applicants' understanding of current law that the term of this patent will be measured from the earliest claimed priority date. The priority claim cancellation is not intended as an admission of whether or not the claimed invention would be entitled to priority, if the priority claim to the November, 1994 application were maintained. The Applicants reserve the right to maintain the same priority claim for subject matter that may be pursued in related applications, such as continuations, continuations-in-part, divisional applications, reissue applications, or the like. It is the Applicants' understanding from prosecution that the subject matter of the allowed claims has been deemed patentably distinct from any subject matter disclosed in art of record, including subject matter disclosed in two U.S. patents issued to Human Genome Sciences (Hu et al., U.S. Patent Nos. 5,932,540 and 5,935,820) that were considered by the Examiner. (One of these patents was cited by the Examiner as a reference under §102(e) and distinguished by the Applicants. See Amendment dated July 24, 2000, at pages 14-18.) Thus, the presence or absence of the priority claim raises no patentability issues.¹

¹ The November, 1994 patent application has issued as U.S. Patent No. 5,776,755. The '755 patent is not prior art under §102(e) because, to the extent the '755 patent discloses or suggests the present invention, the relevant disclosure is a disclosure of the present inventors' own work. Because the relevant portions of the '755 patent constitute the inventor's own work, the relevant filing date of the '755 patent was not "before the invention thereof by the applicant" as required by §102(e). (It is impossible to disclose the inventors' own work before the inventors invented it.)

At page 7, line 25, please delete "Figure 5 shows" and insert --Figure 5A-5C
show--.

At page 7, line 28, please delete "Figure 6 shows" and insert --Figures 6A and
6B show--.

At page 8, line 6, please delete "Figure 9 shows" and insert --Figures 9A
through 9C show--.

At page 8, line 8, please delete "Figure 10 shows" and insert --Figures 10A
and 10B show--.

At page 9, line 21, please delete "Figure 2" and insert --Figures 2A and 2B--.

At page 18, line 16, please delete "Figure 6" and insert --Figures 6A and 6B--.

At page 24, line 30, please delete "Figure 9" and insert --Figures 9A through
9C--.

At page 25, line 6, please delete "Figure 10" and insert --Figures 10A and
10B--.

At page 25, line 11, please delete "Fig. 9" and insert --Figures 9A through
9C--.

At page 26, line 7, please delete "Fig. 9" and insert --Figures 9A through 9C--.

At page 26, line 24, please delete "Fig. 10" and insert --Figure 10A--.

REMARKS

Applicants request entry of the foregoing amendments, which relate solely to
formal matters. These amendments are being presented prior to or concurrently with payment
of the issue fee as required by Rule 312. The amendments do not affect the scope or content of



If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

***NOTE:**

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).

NAME: Ludwig Institute for Cancer Research
ADDRESS: 1345 Avenue of the Americas, New York, NY 10105
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☒ NONPROFIT ORGANIZATION

NAME: _____
ADDRESS: _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Heikki Lampi

TITLE OF PERSON OTHER THAN OWNER: President

ADDRESS OF PERSON SIGNING: Viikinkaari 8 A, FIN-00710 Helsinki, Finland

SIGNATURE: _____

Date

27. Feb. 1997



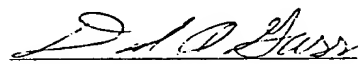
01-05-01

B

PATENT

Attorney Docket No.: 28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)	I hereby certify that this paper is being
)	deposited with the United States Postal
Serial No: 08/510,133)	Service, in an envelope addressed to the:
)	Commissioner for Patents, Box Issue
Filed: August 1, 1995)	Fee, Washington, D.C. 20231, utilizing
)	the "Express Mail Post Office" under
Title: Flt4 LIGAND AND METHODS)	Mailing Label No. EM578442455US on
OF USE)	this date:
)	
Allowed: October 3, 2000)	January 3, 2001
)	
Batch No.: T33)	
)	David A. Gass
Group Art Unit: 1647)	
)	
Examiner: C. Saoud)	

AMENDMENT AFTER ALLOWANCE PURSUANT 37 C.F.R. § 1.312

Commissioner for Patents
Box Issue Fee
Washington, D.C. 20231

Dear Sir:

Please amend this application as follows:

OK to Enter

AMENDMENTS

In the Specification:

At page 1, line 2, please delete the following priority claim, which had been introduced by way of a Preliminary Amendment dated August 12, 1996:

"This application is a continuation-in-part of U.S. Patent Application Serial No. 08/340,011, filed November 14, 1994."


At page 7, line 19, please delete "Figure 2 shows" and insert --Figures 2A and 2B show--.



PATENT
Attorney Docket No.: 28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)	I hereby certify that this paper is being
Serial No: 08/510,133)	deposited with the United States Postal
Filed: August 1, 1995)	Service, in an envelope addressed to
Title: Flt4 LIGAND AND)	the: Commissioner for Patents, Box
METHODS OF USE)	Issue Fee, Washington, D.C. 20231,
)	utilizing the "Express Mail Post
)	Office" under Mailing Label No.
)	EM578442455US on this date:
Allowed: October 3, 2000)	
Batch No.: T33)	January 3, 2001
Group Art Unit: 1647)	
Examiner: C. Saoud)	


David A. Gass

TRANSMITTAL OF FORMAL DRAWINGS

Commissioner for Patents
Box Issue Fee
Washington, D.C. 20231

Attention: Official Draftsman

Sir:

In response to the requirement made in the notice of allowability dated October 3, 2000, the applicants, through their undersigned attorney, submit herewith 16 sheets of formal drawings (Figures 1-12).

Because Figures 2, 9 and 10 are now each depicted on multiple sheets, and Figure 6 depicted as Figure 6A and Figure 6B on one sheet, the formal drawings are accompanied by an amendment after allowance which updates cross-references to the Figures.

This application was allowed October 3, 2000. The issue fee is being transmitted today under separate cover. Acceptance of the submitted formal drawings is solicited.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By



David A. Gass
Registration No. 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

January 3, 2001

6221839

1 / 16

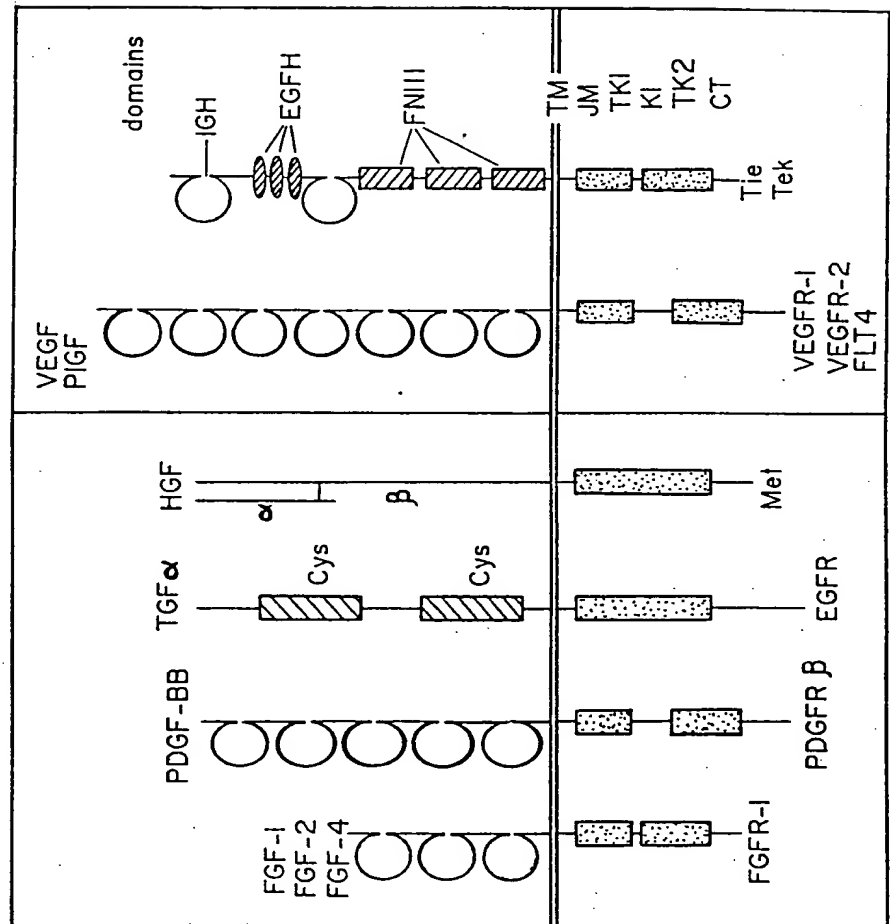


FIGURE 1

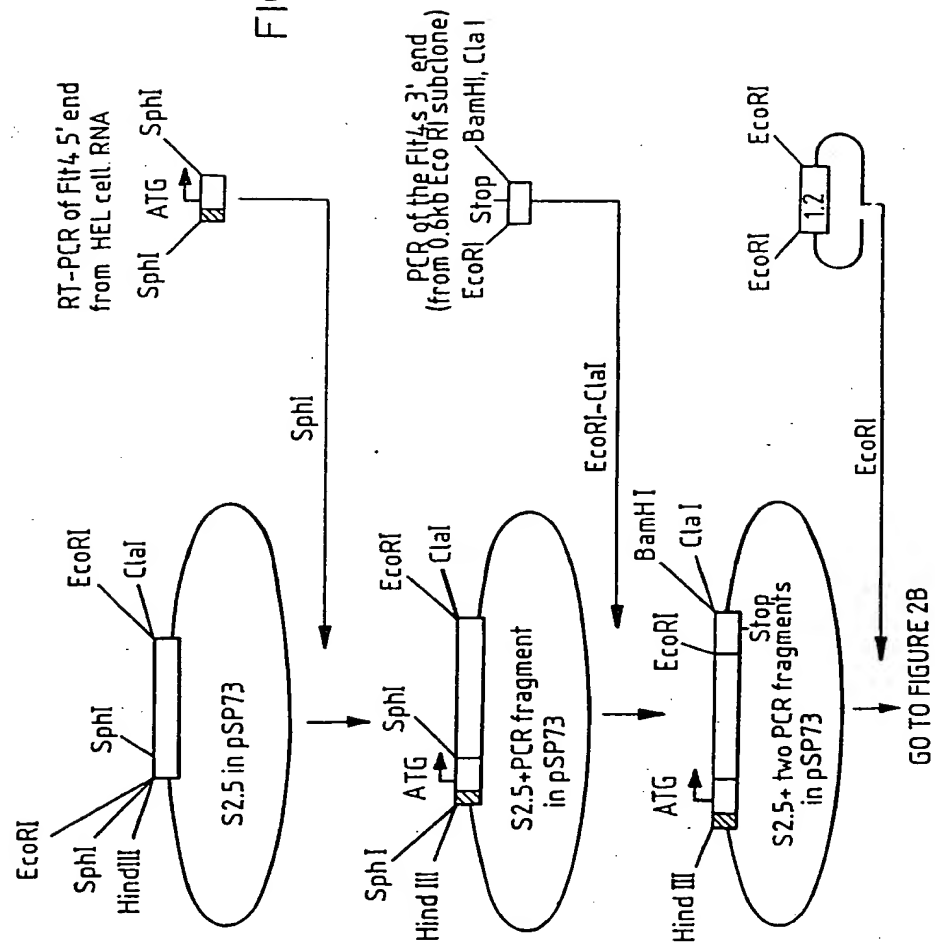


FIGURE 2A

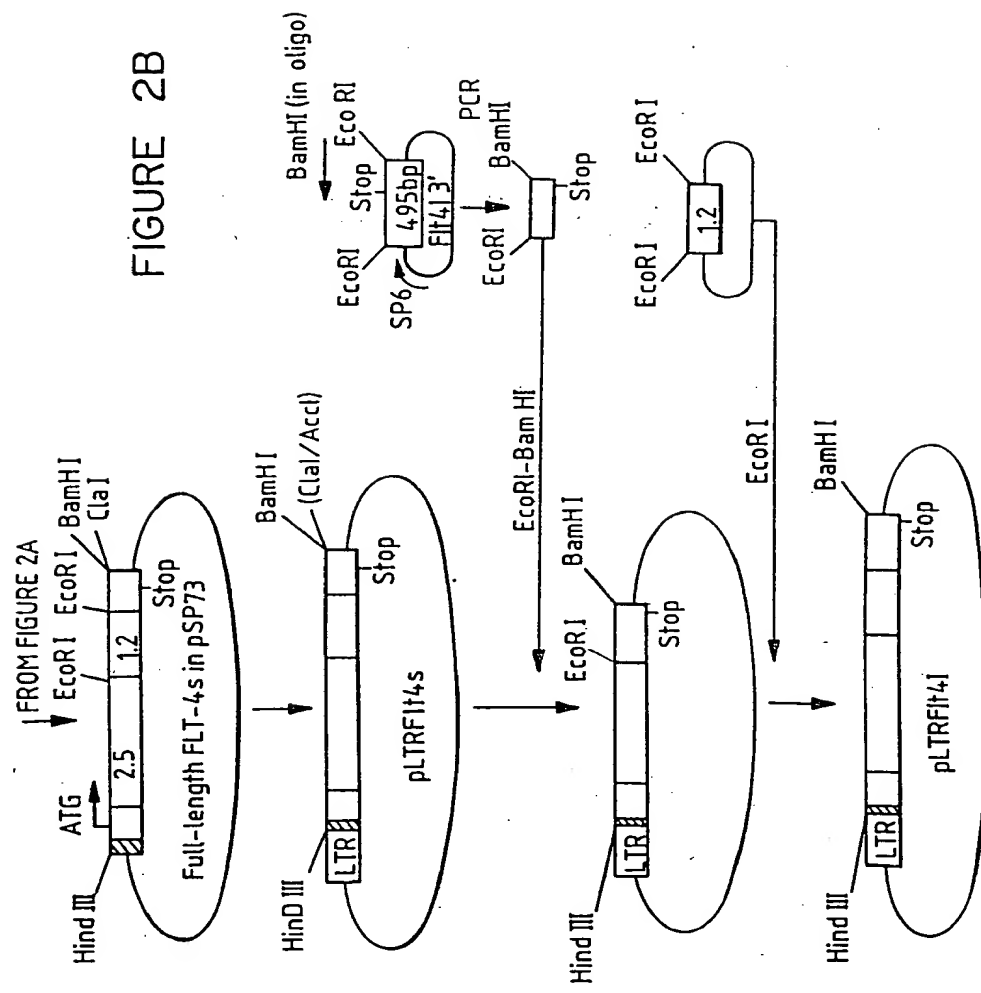


FIGURE 2B

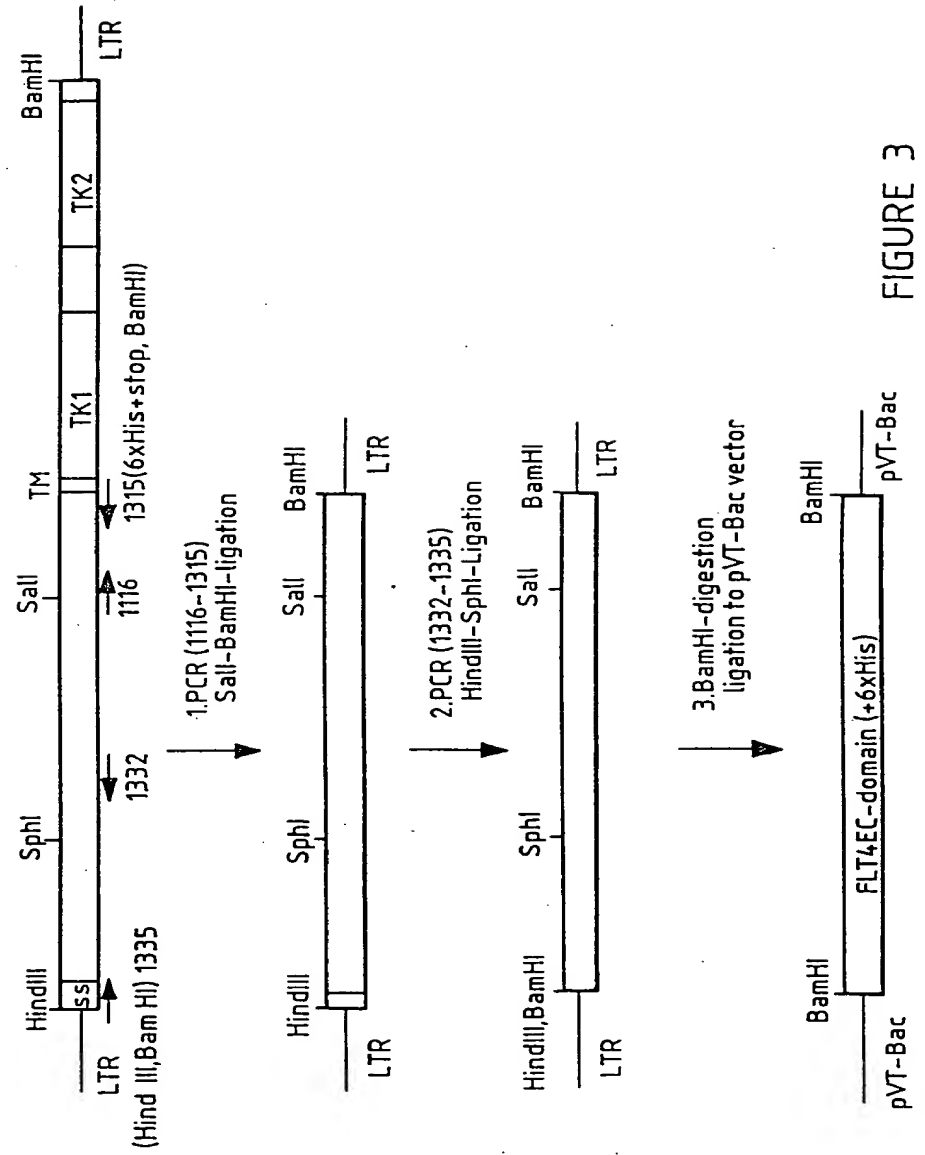


FIGURE 3

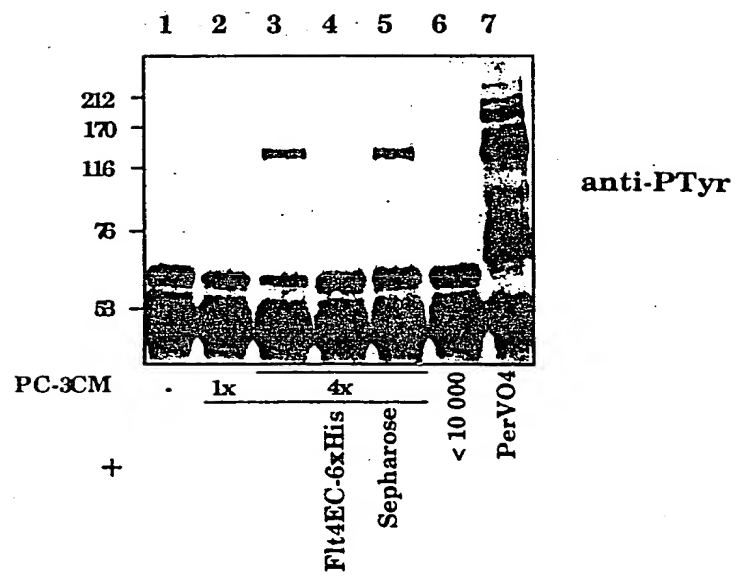


FIGURE 4

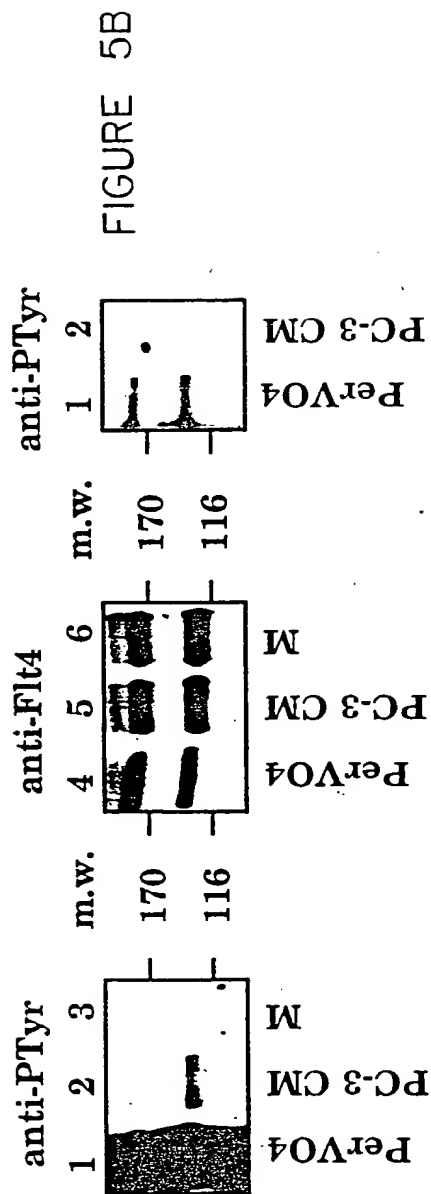


FIGURE 5A

anti-PTyr

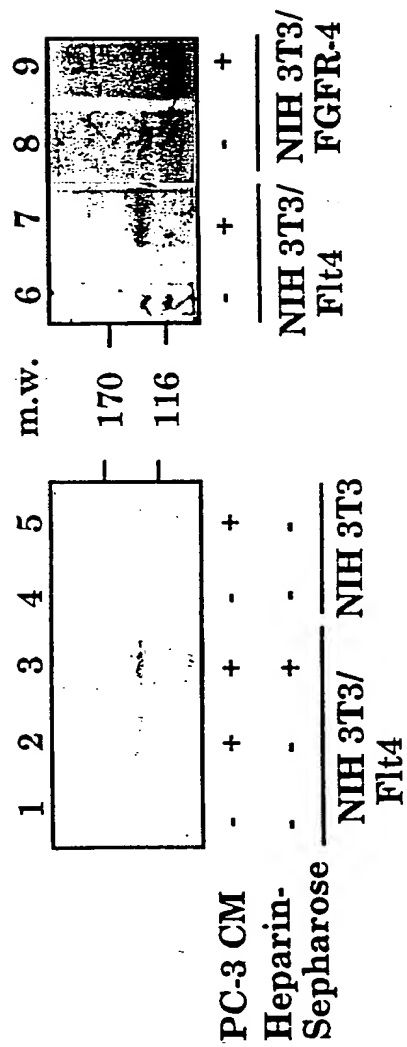


FIGURE 5C

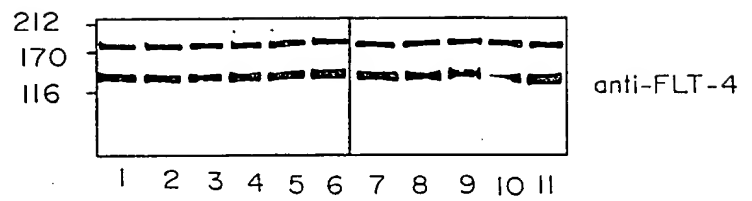


FIGURE 6A

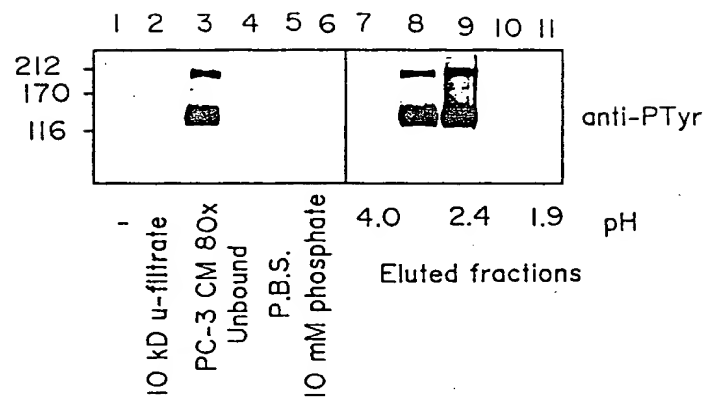


FIGURE 6B

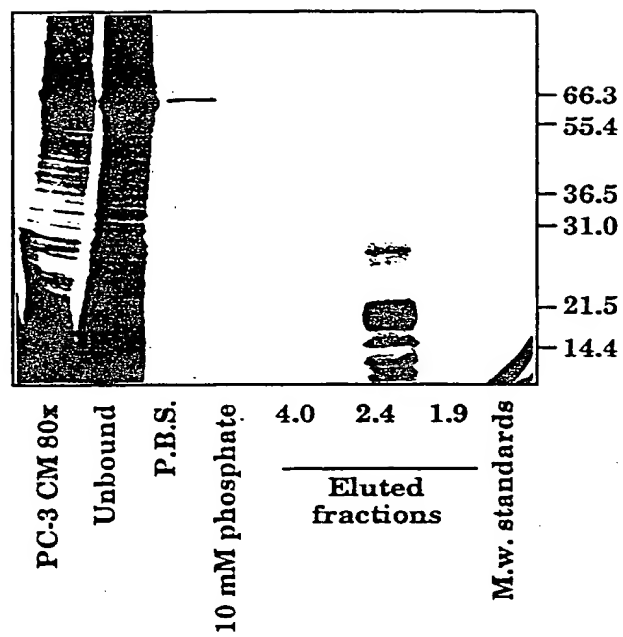


FIGURE 7

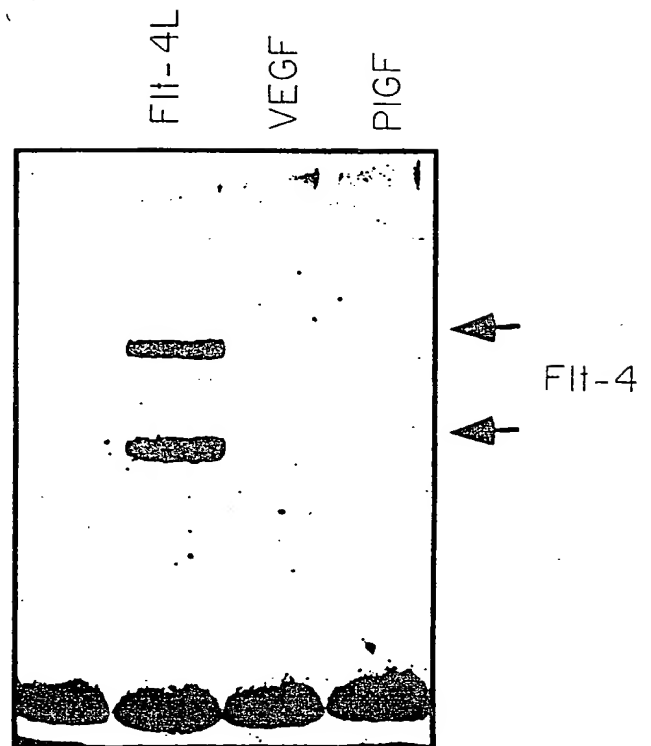


FIGURE 8

MetThrValLeuTyrProGluTyr
 GAGCAGTTACGGTCTGTGTCCAGTGTAGATGAACATCATGACTGTACTCTACCCAGAATAT
 10 30 50

TrpLysMetTyrLysCysGlnLeuArgLysGlyGlyTrpGlnHisAsnArgGluGlnAla
 TGGAAAAATGTACAAAGTGTCTAGCTAAGGAAAGGAGGCTGGCAACATAACAGAGAACAGGCC
 70 90 110

AsnLeuAsnSerArgThrGluGluThrIleLysPheAlaAlaHisTyrAsnThrGlu
 AACCTCAAACTCAAGGACAGAGAGACTATAAAATTTGCTGCAGCACATTATAATACAGAG
 130 150 170

IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys
 ATCTTGAAAAAGTATTGATAATGAGTGGAGAAAGACTCAATGCATGCCACGGGAGGTGTGT
 190 210 230

IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProProCysVal
 ATAGATGTGGGGAAGGAGTTTGGAGTCGCGACAAACACCTTCTTTAAACCTCCATGTGTG
 250 270 290

SerValTyrArgCysGlyGlyCysAsnSerGluGlyLeuGlnCysMetAsnThrSer
 TCCGCTACAGATGTGGGGTTGCTGCAATAGTGAGGGGCTGCAGTGCATGAACACCCAGC
 310 330 350

FIGURE 9A

ThrSerTyrLeuSerLysThrLeuPheGluIleThrValProLeuSerGlnGlyProLys
 ACCAGCTACCTCAGCAAGACGTTATTGAAATTACAGTGCCTCTCTCAAGGCCCCAAA
 370 390 410
 ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal
 CCAGTAAACAATCAGTTTGGCCAATCACACTTCCTGCCGATGCATGCTAAACTGGATGTT
 430 450 470
 TyrArgGlnValHisSerIleIleArgArgSerLeuProAlaThrLeuProGlnCysGln
 TACAGACAAAGTTTCATTCCATTATTAGACGTTCCCTGCCAGCAACACTACCACAGTGTCTCAG
 490 510 530
 AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu
 GCAGCHACAAGACCTGCCCCCAATTAATCATGTGGAATAATCACATCTGCAGATGCCTG
 550 570 590
 AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspSerThrAspGlyPheHis
 GCTCA3GAAGATTTTATGTTTTCCTCGGATGCTGGAGATGACTCAACAGATGGATTCCAT
 610 630 650
 AspIleCysGlyProAsnLysGluLeuAspGluGluThrCysGlnCysValCysArgAla
 GACATCTGTGGACCAACAAGGAGCTGGATGAAGAGACCTGTCTGTGTCTGTCTGCAGAGCG
 670 690 710

FIGURE 9B

GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys
GGGCTTCGGCCTGCCAGCTGTGGACCCCAAGAACTAGACAGAAACTCATGCCAGTGT
730 750 770

ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAsn
GTCTGTAAAAACAACCTCTTCCCAGCCAATGTGGGGCCCAACCGAGAATTTGATGAAAAAC
790 810 830

ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys
ACATGCCAGTGTGTATGTAAAGAAGACCTGCCCCCAGAAATCAACCCCTAAATCCTGGAAAAA
850 870 890

CysAlaCysGluCysThrGluSerProGlnLysCysLeuLysGlyLysLysPheHis
TGTGCCTGTGAATGTACAGAAAGTCCACAGAAATGCTTGTAAAGGAAAGAGTCCAC
910 930 950

HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro
CACCAACATGCAGCTGTTACAGACGGCCATGTACGAACCGCCAGAGGCTTGTGAGCCA
970 990 1010

GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln
GGATTTTCATATAGTGAAGAAGTGTGTCGTTGTGTCCCTTCATATTGAAAAAGACCCACAA
1030 1050 1070

MetSerEnd
ATGAGCTAAGATTGTACTGTTTCCAGTTTCATCGATTTTCTATTATGGAAAACTGTGTTG
1090 1110 1130

			50
1			
FDGF-A	.MRTWACLLL	LGGYLAHAL	AEEAIEPREL IERLARSQIH SIRDQLRLE
FDGF-B	MNRCWA.LFL	SLCCYLRLVS	AEGDPIPEEL YEMLSDHSIR SFDDLQRLH
FlGF	MP VMRLFPCFLQ LLAGLAL...
VEGFMNFLLSWH WSLALLLYLH
FLT4-L	MTVLYPEYWK MYKCQLRKGG
	51		100
FDGF-A	IDSVGAEDAL	ETSLRAHGSH	AINHVPEKRP VPIRRKRSI.EEAIP
FDGF-B	GDP.GEEDGA	ELDLNMTRSH	SGGELES... .LARGRRSLG SLTIAEPAMI
FlGF	PAVPPQWAL	SA.....	NGSSEVEVV P.FQEVWG.. ..R
VEGF	HAKWSQAAPM	AE.....	GGQNHHEVV K.FMDVYQ.. ..R
FLT4-L	WQHNREQANL	NSRTEETIKF	AAAHYNTEIL KSIDNEWR.. ..K
	101		150
FDGF-A	AVCKTRTVIY	EIPRSQVDPT	SANFLIWPCC VEVKRCIGCC NTSSVKCQPS
FDGF-B	AECKTRTEVF	EISRRLLDRT	NANFLVWPPC VEVQRCSGCC NNRNVQCRPT
FlGF	SYCRALERLV	DVVSEY..PS	EVEHMFSPSC VSLLRCHGCC GDENLHCVPV
VEGF	SYCHPIETLV	DIFQEY..PD	EIEYIFKPPC VPLMRCGGCC NDEGLECVPT
FLT4-L	TQCMPREVCI	DVGKEF..GV	ATNTFFKPPC VSVYRCGGCC NSEGLQCMNT
	151		200
PDGF-A	RVHRSVKVA	KVEYVRKKPK	LKEVQVRLEE HLEACI.... AT.....
PDGF-B	QVQLRPVQVR	KIEIVRKKPI	FKKATVTLED HLAACKI.... ETVAAARPVT
FlGF	ETANVTMQLL	KIRSG..DRP	.SYVELTFSQ HVRCECRPLR EKMKPERC..
VEGF	EESNITMQIM	RIKPH..QGQ	.HIGEMSFLQ HNKCECRPKK DRARQENP..
FLT4-L	STSYLSKITLF	EITVPLSQGP	.KPVTTISFAN HTSQRGMSKL DVYRQVHSII

FIGURE 10A

201	PDGF-A	..SNLNPDR	EEETDVR	250
	PDGF-B	RSPGGSQEQ	AKTPQTRVTI	RTVRVRRPPK	GKHKFKHTH	DKTALKETLG
	PlGF	GDAVPRR
	VEGF	CGPCSERKH	LFVQDPQTK	CCKNTDSRC	KARQLELNER
	FLT4-L	RRSLPATLPQ	CQAANKTCPT	NYMNNNHICR	CLAQEDFMFS	SDAGDDSTDG
		251				300
	PDGF-A
	PDGF-B	A.....
	PlGF
	VEGF	TCRCDKPRR
	FLT4-L	FHDICGPNKE	LDEETCQCVC	RAGLRPASCG	PHKELDRNSC	QCVCKNKLFP
		301				350
	PDGF-A
	PDGF-B
	PlGF
	VEGF
	FLT4-L	SQCGANREFD	ENTCQCVCCKR	TCPRNQPLNP	GKCACTES	PQCLLLGKK
		351				395
	PDGF-A
	PDGF-B
	PlGF
	VEGF
	FLT4-L	FHHQTCSCYR	RPCTNRQKAC	EPGFSYSEEV	CRCVPSYWKR	PQMS

FIGURE 10B

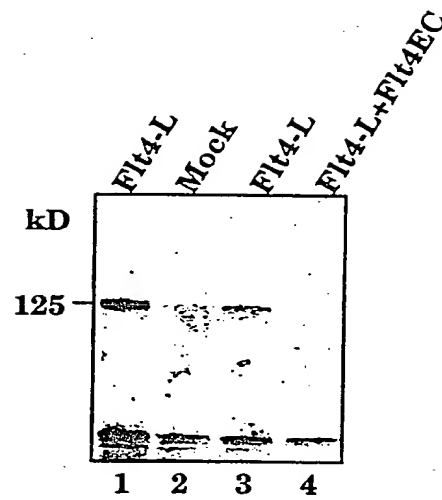


FIGURE II

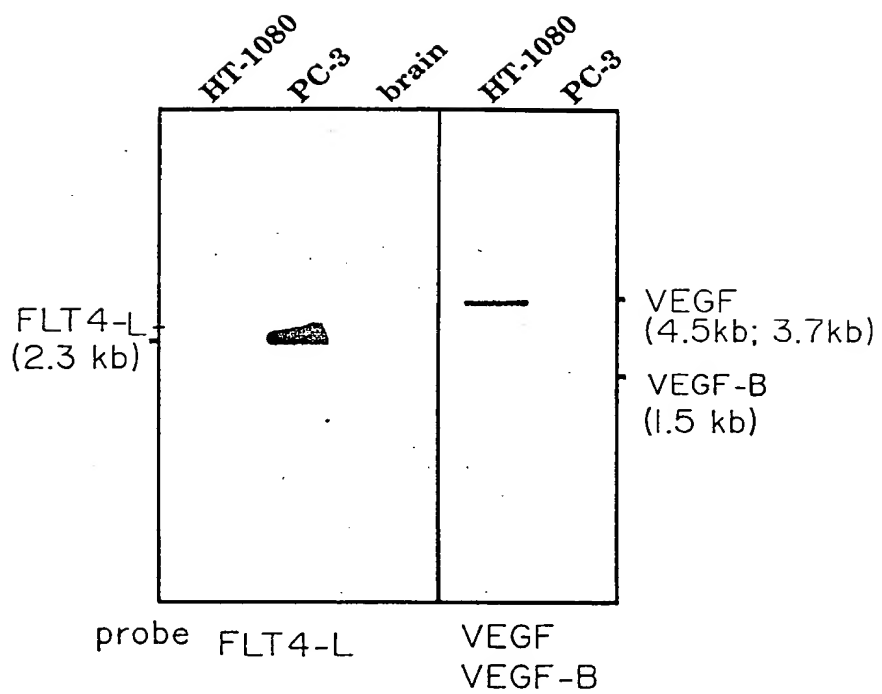
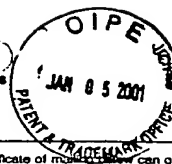


FIGURE 12

PART B—ISSUE FEE TRANSMITTAL

Complete and mail this form, together with applicable fees, to: **Box ISSUE FEE**
Assistant Commissioner for Patents
Washington, D.C. 20231



MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE. Blocks 1 through 4 should be completed where appropriate. All further correspondence including the Issue Fee Receipt, the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark-up with any corrections or use Block 1)

Marshall, O'Toole, Gerstein, Murray & Borun
233 South Wacker Drive
63rd Floor
Sears Tower
Chicago, Illinois
60606

Note: The certificate of mailing can only be used for domestic mailings of the Issue Fee Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing.

Certificate of Mailing

I hereby certify that this Issue Fee Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Box Issue Fee address above on the date indicated below.

David A. Gass (Depositor's name)

(Signature)

JANUARY 3, 2001 (Date)

APPLICATION NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
08/510,133	08/01/95	029	SAOUD, C	12/47 10/03/00
First Named Applicant	ALITALCO, 35 USC 154(d) term ext. 0 Days.			

TITLE OF INVENTION THE LIGAND AND METHODS OF USE
F1+4

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
3 28119/32863 28967	514-012.000	T33	UTILITY	NO	\$1240.00	01/03/01

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). Use of PTO form(s) and Customer Number are recommended, but not required.

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" indication form PTO/SB/47) attached.

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. Marshall, O'Toole,
2. Gerstein, Murray
3. & Borun.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)
PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the PTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE Helsinki University Licensing Ltd. OY;
Ludwig Institute for Cancer Research

(B) RESIDENCE: (CITY & STATE OR COUNTRY) Helsinki, Finland; New York, USA

Please check the appropriate assignee category indicated below (will not be printed on the patent)

- ☐ Individual ☒ corporation or other private group entity ☐ government

4a. The following fees are enclosed (make check payable to Commissioner of Patents and Trademarks):

- ☒ Issue Fee
☒ Advance Order - # of Copies 4

4b. The following fees or deficiency in these fees should be charged to:

DEPOSIT ACCOUNT NUMBER 13-2855
(ENCLOSE AN EXTRA COPY OF THIS FORM)

- ☒ Issue Fee
☐ Advance Order - # of Copies

The COMMISSIONER OF PATENTS AND TRADEMARKS IS requested to apply the Issue Fee to the application identified above.

(Authorized Signature)

(Signature)

(Date)

JAN 03, 2001

NOTE: The Issue Fee will not be accepted from anyone other than the applicant, a registered attorney or agent, or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending on the needs of the individual case. Any comments on the amount of time required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND FEES AND THIS FORM TO: Box Issue Fee, Assistant Commissioner for Patents, Washington D.C. 20231

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMIT THIS FORM WITH FEE

01/04/2001 08:51:13
01/04/2001 08:51:13
01/04/2001 08:51:13

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 1994

Application or Docket Number

510133

CLAIMS AS FILED - PART I

(Column 1)		(Column 2)
FOR	NUMBER FILED	NUMBER EXTRA
BASIC FEE		
TOTAL CLAIMS	12	minus 20 =
INDEPENDENT CLAIMS	3	minus 3 =
MULTIPLE DEPENDENT CLAIM PRESENT		

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
	365.00	OR		730.00
x\$11=		OR	x\$22=	
x38=		OR	x76=	
+120=		OR	+240=	
TOTAL		OR	TOTAL	730

CLAIMS AS AMENDED - PART II

(Column 1)		(Column 2)	(Column 3)
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	19	Minus	20 =
Independent	3	Minus	3 =
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x38=		OR	x76=	
+120=		OR	+240=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

(Column 1)		(Column 2)	(Column 3)
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	20	Minus	20 = 0
Independent	2	Minus	3 = 0
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x38=		OR	x76=	
+120=		OR	+240=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

(Column 1)		(Column 2)	(Column 3)
AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	29	Minus	23 = 6
Independent	4	Minus	3 = 1
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=	54	OR	x\$22=	<
x38=	39	OR	x76=	
+120=		OR	+240=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
 ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
 *** If the Highest Number Previously Paid For IN THIS SPACE is less than 3, enter "3."
 The Highest Number Previously Paid For (Total or Independent) is the highest number found in the appropriate box in column 1.

[illegible][illegible][illegible][illegible]

[illegible]
